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Journal of the Fisheries Research Board of Canada

Annual Life Cycle and Production of the Chaetognath *Sagitta elegans* in Bedford Basin, Nova Scotia[1]

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Sameoto, D. D. 1973. Annual life cycle and production of the chaetognath *Sagitta elegans* in Bedford Basin, Nova Scotia. J. Fish. Res. Board Can. 30: 333–344.

In Bedford Basin, Nova Scotia, *Sagitta elegans* has two main generations per year, one in the spring and one in the fall. The biomass of *S. elegans* per 100 m^3 of water was an order of magnitude greater in the Basin than that reported previously for St. Margaret's Bay, N.S. The growth rate and the number of degree days needed to mature were similar to those found for *S. elegans* in St. Margaret's Bay. Annual turnover rate for *S. elegans* in the Basin was 5.6 and yearly production was estimated at 27.0 kcal per year or 9.0% of the yearly copepod production. It was calculated that the species used 90.4 kcal per m^2 per year in respiration. The percentage of the total yearly production of copepods consumed by *S. elegans* was estimated at 36%.

Sameoto, D. D. 1973. Annual life cycle and production of the chaetognath *Sagitta elegans* in Bedford Basin, Nova Scotia. J. Fish. Res. Board Can. 30: 333–344.

Dans le bassin Bedford, Nouvelle-Ecosse, *Sagitta elegans* a deux générations principales par année, une au printemps et une autre en automne. La biomasse de *S. elegans* par 100 m^3 d'eau dans le bassin a un ordre de grandeur plus grand que celui déjà signalé dans la baie St. Margaret, Nouvelle-Ecosse. Le taux de croissance et le nombre de degrés-jours nécessaires à la maturation sont semblables à ceux observés chez *S. elegans* de la baie St. Margaret. Le taux de renouvellement annuel de *S. elegans* dans le bassin est de 5.6, et la production annuelle estimée à 27.0 kcal ou 9.0% de la production annuelle de copépodes. On calcule que l'espèce utilise 90.4 kcal par m^2 par année pour la respiration. On estime à 36% le pourcentage de la production totale annuelle de copépodes consommés par *S. elegans*.

Received August 18, 1972

[1]Bedford Institute of Oceanography Contribution.

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REEVE (1968) suggested that chaetognaths were the primary carnivores in the marine environment. This conclusion was partially supported by Sameoto (1971a and 1972), who found that, during the winter in St. Margaret's Bay, Nova Scotia, the *Sagitta elegans* biomass equalled that of the copepods and estimated they consumed up to 50% of the winter's production of copepods but only 1% of the total estimated yearly copepod production.

This paper reports the study on the population of *S. elegans* in Bedford Basin, N.S., which is only 12 miles from St. Margaret's Bay, for which comparable data on the basic production system are available (Sameoto 1971a). It was known from the work of Zo (1969) that the population structure of *S. elegans* in the Basin was less complicated than that found in the Bay and that the biomass of the species was much greater in the Basin (Sameoto 1971c). This provided an opportunity for a comparison to be made between the two populations and thus formed the first step in an assessment of the environmental situation in determining the productivity and impact on the pelagic community of an important invertebrate predator.

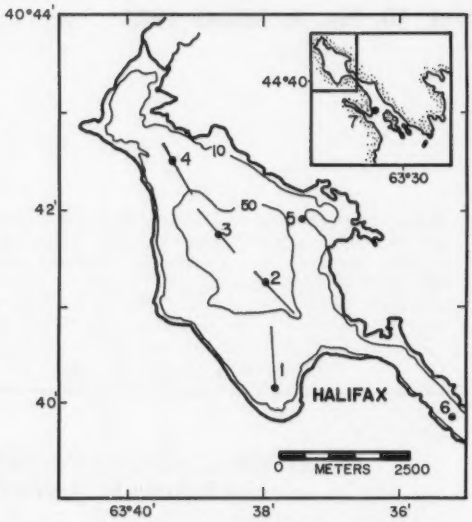


FIG. 1. Sampling stations in Bedford Basin and Halifax Harbor. Lines represent transects for the horizontal samples.

Materials and Methods

STUDY AREA

Bedford Basin is a marine basin 70 m deep, with a surface area of 17 km^2 and a volume of 5×10^8 m^3. It is separated from the sea by a channel, 10 km long and 400 m wide at the narrowest part, which constitutes a sill 20 m deep. Intrusions of offshore water occur throughout the year with some degree of mixing. However, in the summer these are insufficient to prevent the gradual depletion of oxygen to 48% saturation (Platt et al. 1970).

SAMPLING

Zooplankton samples were taken at five stations inside and two stations outside the Basin (Fig. 1). Stations 1–4 were sampled from October 14, 1969, to June 23, 1970, at which time Station 2 was dropped and Station 5 added. This was done because of the similarity in the samples taken at Stations 2 and 3. Stations 6 and 7 were sampled from February 18, 1971, to June 29, 1971.

Horizontal samples were taken from October 14, 1969, to January 7, 1970, with a modified version of the Icelandic highspeed sampler (Sameoto 1971b). Tows were made at 4 kn along 1000-m transects at Stations 1-4 (Fig. 1). Samples were taken simultaneously at depths of 2, 20, and 40 m at Stations 2 and 3 from October 14 to June 7, 1970. After June, samples were taken at depths of 2, 20, 35, and 50 m at Station 3. The position of the stations and the length of the tows on the transects were determined by radar.

Oblique samples from the bottom to the surface were taken from all stations with a metered #6 (233 μ mesh) $\frac{3}{4}$-m ring net and a #20 (76 μ) $\frac{1}{2}$-m ring net. The nets were hauled at a speed of 1 m/sec.

All sampling was done after dark.

TREATMENT OF SAMPLES

The samples were analyzed within 1 week of collection. *S. elegans* over 5 mm in length were removed, counted, and weighed after blotting on filter paper. All other species of macrozooplankton were removed. The remaining sample, consisting mainly of copepods and *S. elegans* less than 5 mm in length, was split a number of times depending on the size of the sample. Small *S. elegans* and *S. elegans* eggs were counted in one of the split portions of the sample. Free water was removed from the entire sample of copepods by vacuuming over a 30 μ filter until water ceased to drip from the funnel. The sample was then weighed to obtain a biomass estimate.

The method used to stage the animals and analyze the different modes of the *S. elegans* population was that used by Sameoto (1971).

All regression equations were calculated by the least squares methods.

The data for the yearly temperature curve (Fig. 2) were calculated by integrating the temperatures from 1 to 70 m at Station 2 by using readings from depths of 1, 5, 15, 25, 40, 60, and 70 m. Temperature data was taken from Platt and Irwin (1971) and Sameoto (1971c).

Third stage animals concentrated in the central deep part of the Basin below 30-m depth. This region con-

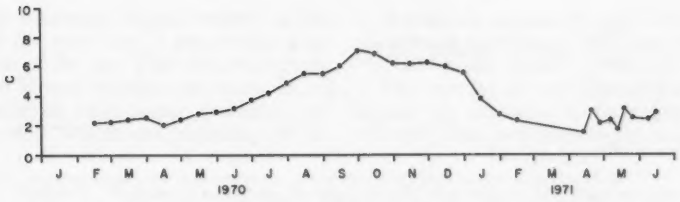


FIG. 2. Yearly mean water temperature of Bedford Basin.

stituted 42% of the volume of the basin. Therefore, the numbers of animals per cubic meter were calculated for the central and outer stations separately. The deep station values were then multiplied by 0.42 and the shallow station values by 0.58. The two values were then added to give an estimate of the mean number of animals per cubic meter in the entire Basin. The data given for the Basin are the weighted means for samples taken at Stations 1–5.

The energy used yearly in respiration was calculated for the population of animals over 5-mm length by using respiration equations for *S. elegans* in Bedford Basin taken from Sameoto (1972). The water column was divided into two layers, 0–25-m depth and 25–70-m depth, and the mean temperature of each layer determined. The appropriate equation was chosen to match the temperature and the time of year. Immature animals were mainly found in the upper 25 m of water and the mature animals in water below 25 m. The mean body length was calculated for the immature and mature animals separately and converted to live body weight by the equation given by Sameoto (1971a), and microliters O_2 used per animal were calculated. The total O_2 (in microliters) used by the animals 100 m^{-3} of water in the layers per 24 hr was calculated by multiplying the number of animals 100 m^{-3} by the number of microliters O_2 used by one animal of mean size in 24 hr. The total O_2 consumption 100 m^{-3} 24 hr^{-1} was plotted for each of the dates and the area calculated using a planimeter to give the total number of milliliters O_2 used 100 m^{-3} $year^{-1}$.

Results

The three types of sampling gear gave different results for the various population parameters for *S. elegans*. The #20 net samples had the highest mean numbers m^{-3} of eggs and animals less than 5 mm in length, while the #6 net samples gave slightly higher values than the #20 net samples for the number of animals greater than 5 mm in length 100 m^{-3} and a higher biomass of *S. elegans* 100 m^{-3} (Fig. 3). The biomass estimates for *S. elegans* from the highspeed samples (Fig. 4) were very similar to the values from the #6 net with the exception of June 11 and July 7. On these two dates the values were lower than those from the #6 net.

The biomass of *S. elegans* reached a maximum in early June, 1970, and in the middle of May, 1971, during the first copepod pulse (Fig. 3). After

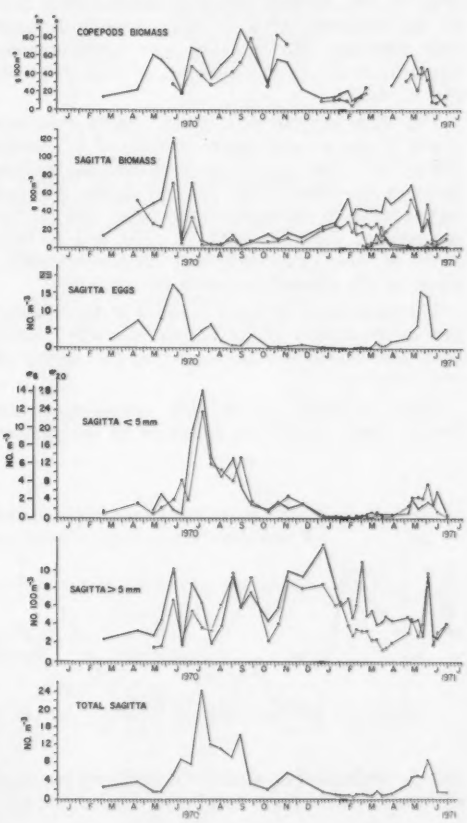


FIG. 3. Seasonal changes in copepod biomass, *Sagitta elegans* biomass, number of *S. elegans* greater and less than 5 mm, number of *S. elegans* eggs, and total number of *S. elegans* (○, #6 ¾-m net and ●, #20 ½-m net mean values of stations 1–5; x, #6 ¾-net at Station 6; △, #6 ¾-m net at Station 7).

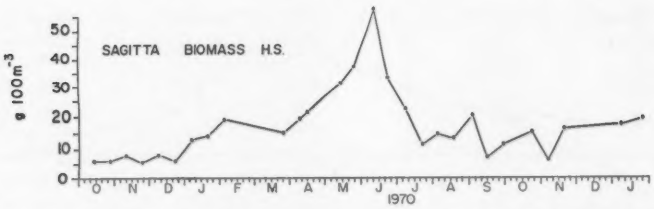


FIG. 4. Biomass of *S. elegans* as estimated by the highspeed horizontal samples.

this period the biomass declined due to the animals dying after reproducing. This was evidenced by the sudden increase in *S. elegans* eggs in May (Fig. 3). The biomass increased steadily from July to the following April or May. During January and February the biomass was approximately the same as that of the copepods as estimated with the #6 net.

The deep central part of the Basin (Stations 2 and 3) had a much higher biomass of *S. elegans* 100 m^{-3} of water than the stations over the shallow parts of the Basin (Fig. 5). This higher biomass was consistent throughout the year except for the samples taken on April 22, 1970, and January 1971. At these times there were no obvious differences in the biomass between the stations.

The biomass at Stations 6 and 7 in the channel and in the mouth of the harbor was much lower than that inside the Basin during the spring of 1971 (Fig. 3).

There appeared to be four spawning pulses for *S. elegans* in 1970 as indicated by the number of eggs present in the #20 net samples (Fig. 3). These occurred in late April, June, early August, and late September. A similar pulse pattern is seen in the total number of *S. elegans* m^{-3} (Fig. 3) with the only difference being a displacement of the peaks later in time.

There were basically two generations, one in the spring and one in the fall. However, each generation had more than one population mode (Fig. 6). Figure 6 shows the size frequency histograms for the three developmental stages and the moving average for the combined size-frequency distribution for all stages along with the calculated normal distribution for each mode on the different sampling dates. It was possible to follow the growth rate of each mode with time as they moved along the length axis with each succeeding sampling date.

The growth rate in terms of dry weight versus age in days was calculated for a number of modes that could be followed with confidence (Table 1). The growth rate increased as the mean water temperature increased in the summer. The mean temperatures are those during the period when the modes were measured, these temperatures are the means from the period the animals were 5 mm long to maturity.

The number of degree days needed by the animals to grow from the egg to maturity was estimated for five modes (Table 2). The mean temperatures in Table 2 are lower than in Table 1 because they include temperatures from the period when the eggs were laid to when the animals were 5 mm long. The temperature during this period was colder than during the latter part of the animals' growth. The mean (780 degree days) fell within the range of degree day values found by Sameoto (1971) for the St. Margaret's Bay population (1077–738 degree days).

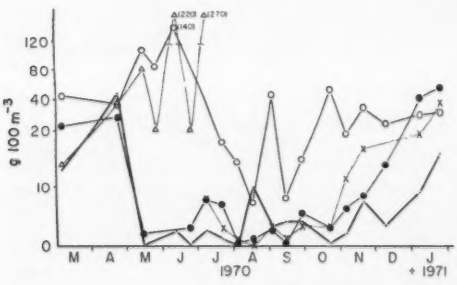


FIG. 5. Biomass of *S. elegans* at five different sampling stations in Bedford Basin. Sample taken with #6¾-m net.

FIG. 6 see pages 338–339

FIG. 6. Frequency–length histograms of development stages of *S. elegans* (stage 1, vertical lines; stage 2, oblique lines to the right; stage 3, oblique lines to the left) and frequency–length curve for the total population with calculated normal curves for population modes. Numbers and letters represent the different population modes. Lettered modes are animals born in 1970 and 1971. Sequence for each column starts at the bottom of the page.

The absolute maximum length reached by the mature stage in the basin was 27 mm, with a maximum mean size of 24 mm. This was significantly lower than the 32 mm absolute maximum length and 28 mm maximum mean length reached by the St. Margaret's Bay population (Sameoto 1971a).

Regression equations were calculated for the number of survivors at different live body weights for six modes (Table 3, Fig. 7).

The number of animals per cubic meter in a mode was calculated by estimating the percentage of the total population the mode represented.

TABLE 1. Regression equations for $\log_{10}$ of dry body weight versus age in days for various modes of *Sagitta elegans*, and mean water temperature during measured growth period.

Mode	Equation	Temperature	Date
A	$\log_{10} Y = -0.55+0.007X$	4.8	June 11–Sept. 14
B	$\log_{10} Y = -0.98+0.007X$	4.8	June 11–Sept. 14
C	$\log_{10} Y = -1.46+0.017X$	6.3	Aug. 3–Nov. 19
D	$\log_{10} Y = -0.94+0.012X$	6.5	Sept. 2–Nov. 19
E	$\log_{10} Y = -0.01+0.010X$	6.4	Sept. 14–Jan. 7
F	$\log_{10} Y = -1.09+0.011X$	5.7	Oct. 22–Jan. 25

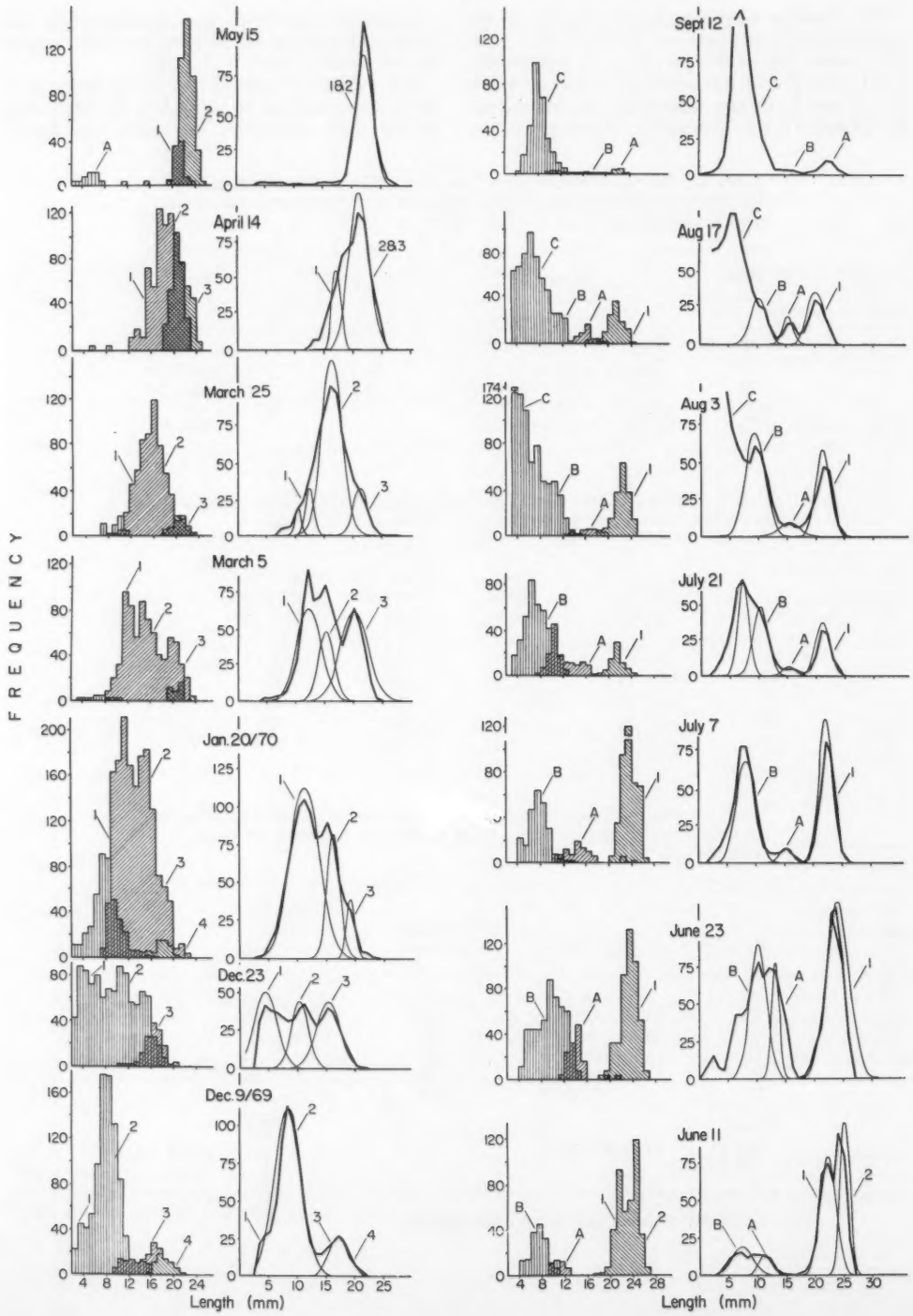
TABLE 2. Estimated times of spawning and maturity of *S. elegans* modes and the mean water temperature during the entire life span of the mode.

Mode	Birth date	Date when mature	Generation length (*days*)	Mean temp (*C*) at which mode grew	Degree days
A	Apr. 15	Oct. 15	183	4.4	807
B	May 15	Nov. 19	188	3.8	730
C	July 15	Dec. 9	147	4.6	672
D	Aug. 15	Apr. 13	241	3.6	861
E	Sept. 15	June 10	233	3.6	833

TABLE 3. Regression equations for numbers of animals per cubic meter versus live body weight modes of the *S. elegans* population. Equations are based on a cohort of 1000.

Mode	Equation	Correlation coefficient	Confidence limits for slope	N[a]
A	$Y = 16X^{-0.86}$	–0.96	–1.09:–0.64	7
B	$Y = 33X^{-0.74}$	–0.99	–0.83:–0.66	6
C	$Y = 57X^{-0.63}$	–0.97	–0.80:–0.45	5
D	$Y = 155X^{-0.49}$	–0.87	–0.81:–0.18	5
E	$Y = 77X^{-0.69}$	–0.90	–1.10:–0.29	5
F	$Y = 182X^{-0.44}$	–0.78	–0.84:–0.05	5
Mean for all modes	$Y = 56X^{-0.68}$			

[a]Number of values used to calculate equation.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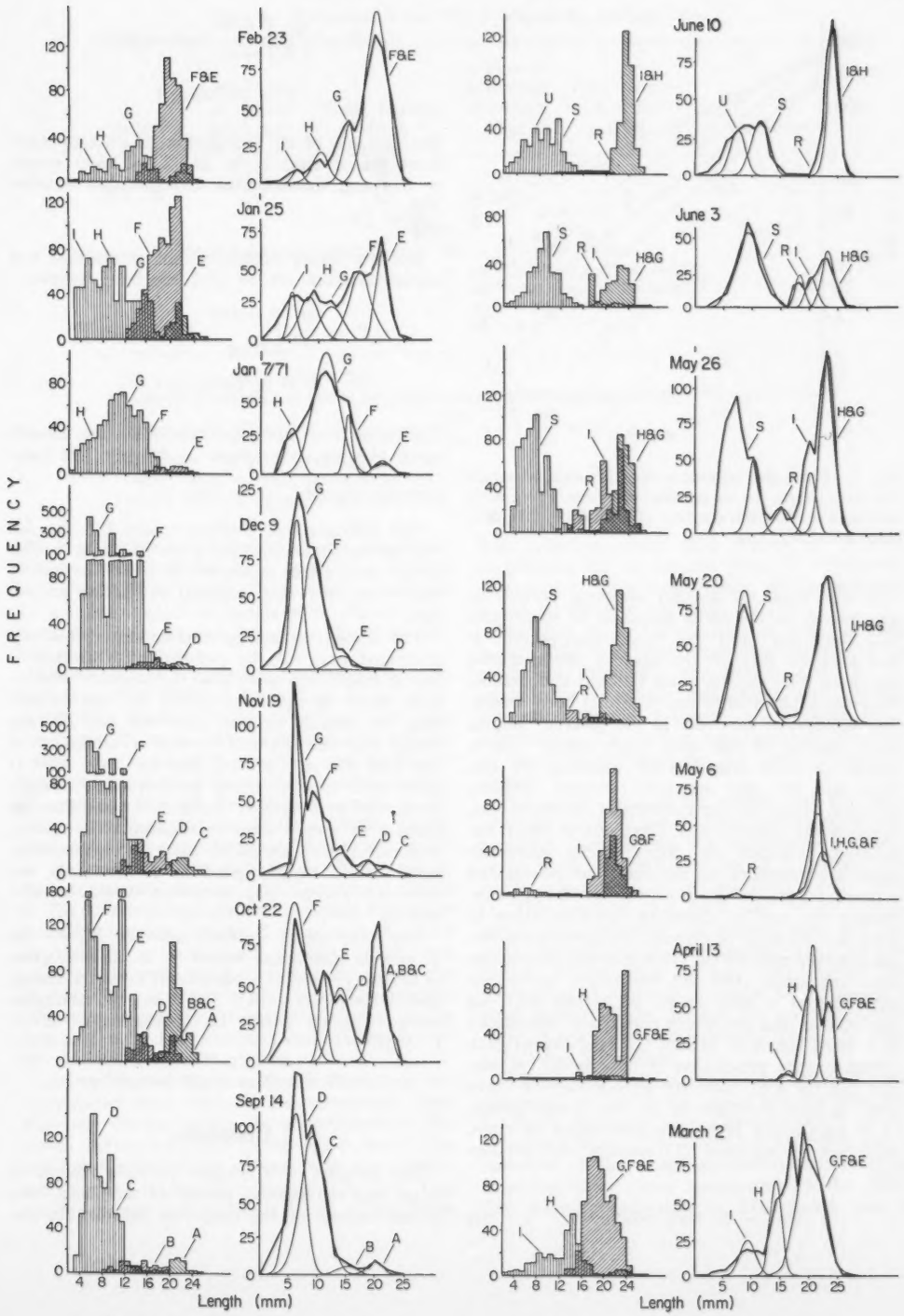
182
April 14
283
March 25
March 5
Jan. 20/70
Dec. 23
Dec. 9/69
Sept 12
Aug 17
Aug 3
July 21
July 7
June 23
June 11
Length (mm)
Length (mm)


FREQUENCY
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Length (mm)
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Jan 25
Jan 7/71
Dec 9
Nov 19
Oct 22
Sept 14
June 10
June 3
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May 6
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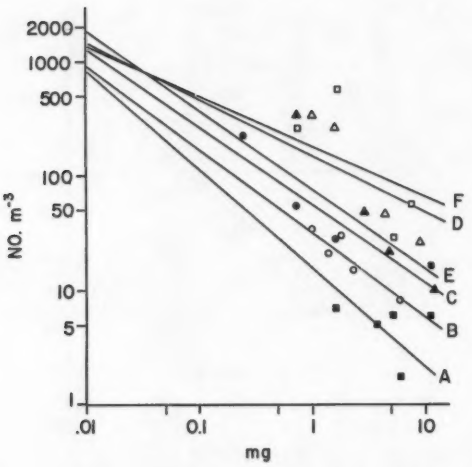

FIG. 7. Calculated regression lines for survival versus live body weight for six population modes. Each mode estimated to start with a cohort of 1000 (■A, ○B, ●C, △D, ▲E, □F).

This was done at different sampling periods as the animals in the mode increased in length and was continued until the mode disappeared. It was assumed that all the eggs at the estimated spawning time for the mode hatched and became the population of survivors of .01 mg body weight. The number of survivors at the different lengths were adjusted so that each mode started with a cohort of 1000 animals each weighing .01 mg. This was the only estimated survival number, all other values for the numbers of survivors were based on the actual counts. There was no significant difference between the slopes of the regression equations; therefore, all the data were pooled and a single regression equation was calculated to represent the survival curve of the population as a whole at all times of the year. Using this equation, the turnover rates for the fall and spring generations were calculated. The fall generation started to reproduce at a body length of 22 mm (12.6 mg live weight) and the spring population reproduced at a mean length of 21 mm (11.3 mg live weight). Therefore, the production (P) of the fall generation was the area under the growth–survival curve (Fig. 7) from a weight of .01 mg (1 mm length) to 12 mg (Ricker 1968). The area under the curve described by equation (1) equalled the production.

$$\text{No.} = 56 \text{ body weight}^{-0.68} \qquad (1)$$

$$P = \frac{56}{3.2} \int_{.01\text{ mg}}^{12\text{ mg}} \text{body weight}^{.32}$$

$$P = 347 \text{ mg m}^{-3}$$

The biomass of the population of animals taken from the survival curve at 12 mg body weight = 124 mg, therefore the turnover rate equalled $\frac{347}{124} = 2.8$

For the spring population the production was calculated from .01 to 11.2 mg body weight.

$$P = \frac{56}{.32} \int_{.01\text{ mg}}^{11.3\text{ mg}} \text{body weight}^{.32}$$

$$P = 339 \text{ mg m}^{-3}$$

The biomass of the population when the animals reach 11.3 mg body weight = 121 mg. The turnover rate equalled $\frac{339}{121} = 2.8$.

The calculated production values for the fall and spring generation are given in Table 4. The spring generation produced 14% of the yearly total while 86% was produced by the fall generation.

The amount of energy used in respiration was calculated only for the population of animals 5 mm in length and over, since the respiration equations given by Sameoto (1972) did not include data on animals smaller than this size. It was found that the population used a minimum of 156 kcal 100 m^{-3} or 45.2 kcal m^{-2} yr^{-1}. This is about twice as much energy as went into the yearly production of *S. elegans* (Table 5). To calculate the amount of energy used in respiration in nature, Sameoto (1972) estimated that the respirometer value should be multiplied by 2. Therefore, the minimum energy used in nature would be 90.4 kcal m^{-2} yr^{-1}.

The total energy ingested annually (C) by the *S. elegans* population would be at least as great as the energy used in production (P) plus the energy used in respiration (R). Therefore, the minimum energy ingested would be approximately C = P + R,

$$C = 29.6 + 90.4 = 120 \text{ kcal m}^{-2} \text{ yr}^{-1}.$$

Discussion

The biomass of *S. elegans* per unit volume of water was significantly greater in the Basin than in the channel or the mouth of Halifax Harbor.

TABLE 4. Production values for *S. elegans* in Bedford Basin.

Date	Mean wt of mature stage (*mg*)	Total Biomass g 100 m^{-3}	% biomass represented by mature stage	Total biomass of mature stage	Production[a]
June 11	12.6	77	97	75	210
Oct. 22	11.3	15	80	12	33
					243

Estimated annual production = 243 + 17[b] = 260 g 100m^{-3}
or 76.5 g m^{-2} yr^{-1} live weight[c]
or 6.3 g m^{-2} yr^{-1} dry weight[d]
or 29.6 kcal m^{-2} yr^{-1}[e]
or 2.4 g C m^{-2} yr^{-1}.[f]

[a]75 × 2.8 (turnover rate) = 210.
[b]Amount of weight loss due to leaching in preservative (Sameoto 1971a).
[c]Mean depth of basin 29 m; therefore, $\frac{100}{29} = 3.4$, $\frac{260}{3.4} = 76.5$ g.
[d]Dry wt = 8.3% live wt (Sameoto 1971a).
[e]1-g dry wt = 4.7 kcal (Sameoto 1971a).
[f]38.5% dry wt is carbon (Sameoto 1971a).

This demonstrated that the *S. elegans* population within Bedford Basin was essentially isolated from the offshore waters. There was no evidence for a sudden export or import of *S. elegans* to or from the Basin as was found in St. Margaret's Bay (Sameoto 1971a).

The biomass of *S. elegans* was an order of magnitude greater in Bedford Basin than in St. Margaret's Bay (Sameoto 1971a). This may have been due in part to less flushing of the Basin by offshore waters than is found in the Bay, and the higher density of copepods in the Basin. The mean numbers of copepods m^{-3} of the common genera in St. Margaret's Bay and in Bedford Basin during 1969 and 1970 were compared. The genera found in the two areas were *Calanus*, *Pseudocalanus*, *Temora*, *Acartia*, *Oithona*, and *Centropages* (Table 6). The data have been matched as closely as possible according to date for the 2 years and the ratio of numbers m^{-3} calculated. Bedford Basin had 1.7–104.7 times as many copepods m^{-3} as did the Bay at different times of the year. The greatest difference in copepod numbers occurred during June and early July, the peak reproductive period for *S. elegans* in both regions.

The population structure in the Basin was less complicated than that in St. Margaret's Bay. The very distinct modes, or subpopulations (Sameoto 1971a), were not found in the Basin. The maximum length of the mature stage was significantly smaller in the Basin than in the Bay. This may have been due to the isolation of the Basin from offshore water that introduced different populations of *S. elegans* into St. Margaret's Bay, which were larger than those ordinarily found in the Bay (Sameoto 1971a).

The first major production of eggs occurred in late May, with each subsequent increase in egg production occurring immediately after an increase in the copepod biomass. A decline in copepod biomass generally followed an increase in *S. elegans* eggs. The declines may have been the result of increased predation by the newly hatched *S. elegans*.

The growth rates of the modes were the same as those in St. Margaret's Bay at the same temperatures, even though the numbers of copepods in the Basin, the principle prey of *S. elegans*, were much greater than in St. Margaret's Bay (Table 6). This suggests that food was not a limiting factor in determining the growth rate, but that it was limited by the water temperature. This conclusion is supported by data that showed that the number of degree days needed by *S. elegans* to reach maturity from the egg was not significantly different from the number found for the St. Margaret's Bay population.

The annual turnover rate of the Basin population was 2 × 2.8 = 5.6, which was less than that for the St. Margaret's Bay population (T.R. = 6.1, Sameoto 1971a). This lower turnover rate was a reflection of the longer generation time resulting from the lower mean temperature in the Basin. The annual production in kilocalories was 10

TABLE 5. Estimated microliters O_2 and total calories used in respiration 100 m^{-3} 24 hr^{-1} by *S. elegans* for one year. Respiration regression equations taken from Sameoto (1972).

Date	Depth	Mean temp (*C*)	Respiration equation	$\overline{X}$ body length (*mm*)	$\overline{X}$ body wt (*mg*)	microliters O_2 animal^{-1} 24 hr^{-1}	% of total Biomass 100 m^{-3}	Biomass (*g 100 m*$^{-3}$)	Milliliters O_2 used by pop. 100 m^{-3} 24 hr^{-1}	Total milliliters O_2 used by pop. 24 hr^{-1}	Total[a] calories 24 hr^{-1}
Dec. 9	0–25	7.0[b]	$Y = 1.55\ x^{.85}$	8	1.2	1.8	77	6.2[c]	9.3	11.7	56.1
	25–60	4.7[b]	$Y = 3.82\ x^{.45}$	17	6.9	9.1	23	1.8[c]	2.4		
Dec. 23	0–25	6.0[b]	$Y = 3.82\ x^{.45}$	10	2.0	5.2	75	4.1[c]	10.7	12.5	60
	25–60	4.7[b]	$Y = 3.82\ x^{.45}$	17	6.9	9.1	25	1.4[c]	1.8		
Jan. 20	0–25	1.6[b]	$Y = 1.76\ x^{.69}$	13	3.7	4.3	94	13.3[c]	15.4	16.3	78.2
	25–60	3.3[b]	$Y = 2.61\ x^{.64}$	19	9.0	10.6	6	.8[c]	.9		
Mar. 5	0–25	1.0	$Y = 1.76\ x^{.69}$	15.7	5.8	5.9	100	24	24.4	24.4	117
	25–60	2.3	$Y = 1.76\ x^{.69}$								
Mar. 25	0–25	1.7	$Y = 1.76\ x^{.69}$	16.1	6.0	6.0	100	15	15.0	15.0	72
	25–60	2.0	$Y = 1.76\ x^{.69}$								
Apr. 14	0–25	2.0	$Y = 1.76\ x^{.69}$	19.8	9.9	8.6	100	38	33	33	158
	25–60	2.0	$Y = 1.76\ x^{.69}$								
May 15	0–25	3.4	$Y = 11.94\ x^{.34}$	6	.6	21.9	15	7.2	262	345	1656
	25–60	2.0	$Y = 3.67\ x^{.56}$	22	12.6	20.7	85	40.8	67		
June 11	0–25	4.0	$Y = 11.94\ x^{.34}$	9	1.6	25.2	3	2.3	36	147	706
	25–60	2.0	$Y = 3.67\ x^{.56}$	23.1	14.1	21.3	97	73.7	111		
June 23	0–25	4.1	$Y = 11.94\ x^{.34}$	10.7	2.4	26.7	47	15.3[c]	170	195	937
	25–60	2.2	$Y = 3.67\ x^{.56}$	23.4	14.5	21.4	53	17.2[c]	25		
July 7	0–25	5.0	$Y = 4.40\ x^{.71}$	7.7	1.1	18.7	17	13.8	233	405	1944
	25–60	2.9	$Y = 11.94\ x^{.34}$	22.5	13.3	34.4	83	67.2	172		
July 21	0–25	6.0	$Y = 4.90\ x^{.71}$	8.6	1.4	20.3	60	6.0	85	90	451
	25–60	3.2	$Y = 3.09\ x^{.71}$	21.5	12	27.3	40	4.0	9.0		
Aug. 3	0–25	7.0	$Y = 5.85\ x^{.68}$	8	1.2	24.0	25	1.7	33.6	44.2	212
	25–60	3.6	$Y = 3.09\ x^{.71}$	23	14	28.6	75	5.2	10.6		
Aug. 17	0–25	7.5	$Y = 5.85\ x^{.68}$	8	1.2	6.6	40	2.1	11.5	14.0	67.2
	25–60	4.0	$Y = 1.50\ x^{.77}$	23	14	11.4	60	3.1	2.5		
Sept. 2	0–25	7.4	$Y = 5.85\ x^{.68}$	6.7	.8	21.3	40	8.4	224	243	1166
	25–60	4.1	$Y = 1.50\ x^{.77}$	19.4	9.4	14.7	60	12.6	19.1		
Sept. 14	0–25	7.6	$Y = 5.85\ x^{.68}$	8.6	1.4	25.3	79	2.9	52.4	53.4	256
	25–60	4.1	$Y = 1.50\ x^{.77}$	20.2	10.4	15.2	21	.7	1.0		
Oct. 22	0–25	8.3	$Y = 1.55\ x^{.85}$	8	1.2	9.1	25	3.7	28.0	41.4	199
	25–60	4.6	$Y = 3.44\ x^{.85}$	21	11.3	13.5	75	11.2	13.4		
Nov. 19	0–25	7.6	$Y = 1.55\ x^{.85}$	9	1.6	10.0	70	11.9	74.4	80.6	387
	25–60	4.6	$Y = 3.82\ x^{.45}$	22	12.6	15.3	30	5.1	6.2		
Dec. 9	0–25	7.0	$Y = 1.55\ x^{.85}$	9	1.6	10.0	85	12.3	76.8	79.3	381
	25–60	4.7	$Y = 3.82\ x^{.45}$	22	12.6	15.3	15	2.1	2.5		

[a]Oxy calorific equivalent of 4.8 used to convert milliliters O_2 to calories.
[b]Temperature taken from 1970 data.
[c]Biomass data taken from high speed plankton samples.

TABLE 6. Mean numbers of copepods per cubic meter of the common genera in Bedford Basin (BB)[a] and St. Margaret's Bay (SMB)[b] for 1970 and 1969, respectively, plus the ratio of copepods per cubic meter in the two regions. Samples were taken with a #6¾-m ring net.

BB		SMB		Ratio BB
1970	Total	1969	Total	SMB
Mar. 5	2644	Mar. 25	146	18.1
Apr. 22	4666	Apr. 15	427	9.9
May 14	6552	May 5	1107	5.9
June 11	7015	June 14	67	104.7
June 23	5571	June 19	97	57.4
July 7	19589	July 7	328	59.7
Aug. 3	12340	Aug. 7	7043	1.7
Sept. 2	18438	Aug. 26	5753	3.2
Sept. 14	25437	Sept. 8	7030	3.6
Sept. 28	32665	Oct. 6	5095	6.4
Nov. 5	16486	Nov. 5	2908	5.7
Dec. 9	5394	Dec. 1	1711	3.1

[a]Data obtained from Sameoto (1971c).
[b]Data obtained from R. J. Conover (Personal communication).

times as great as that in St. Margaret's Bay because of the much higher biomass of *S. elegans* in the Basin throughout the year.

The energy used annually in respiration by the Basin population was twice as great relative to the yearly production as that used by the population of *S. elegans* in St. Margaret's Bay. This was due to the proportionately larger percentage of small immature animals in the Basin population, as compared to the Bay, and the smaller size at maturity of the fall generation in the Basin. Small animals used more O_2 per unit weight than large animals (Sameoto 1972).

The annual primary production of Bedford Basin in 1970 was found to be 220 g C m^{-2} yr^{-1} (T. R. Platt personal communication). If it is assumed that 10% of the primary production goes into copepod production, a figure generally accepted (Slobodkin 1968), then the copepods produced 22 g C m^{-2} yr^{-1}, or 329 kcal m^{-2} yr^{-1}. The yearly production of *S. elegans* was found to be 29.6 kcal m^{-2} yr^{-1}, which is 9.0% of the yearly copepod production. The minimum percentage of the copepod production consumed by *S. elegans* would be the production energy plus respiration energy which was

$$\frac{29.6 + 90.4}{329} = 36\%.$$

S. elegans thus appears to be one of the major copepod predators in the Basin and a much more important predator in the Basin than it is in St. Margaret's Bay, where Sameoto (1972) estimated it consumed between 0.07 and 1.1% of the annual copepod production. This was based on the assumption that the copepod production was 10% of the primary production of phytoplankton and seaweeds. If only phytoplankton went into copepod production, it would produce 300 kcal m^{-2} yr^{-1} and the *S. elegans* population would have consumed between 2.2 and 3.3% of the yearly copepod production in St. Margaret's Bay.

The value of 9.0% is close to the widely accepted value of 10% for energy transferred from one trophic level to the next highest. This leaves little energy for the other copepod predators such as medusae and ctenophores, both of which were abundant in the Basin at various times of the year (Sameoto 1971c). If these alternate predators require a similar prey production to support them, it would appear that 10% energy transfer from primary production to copepods is too low. If this is the case, it still does not alter the basic conclusion that *S. elegans* is the major copepod predator in Bedford Basin.

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A Quantitative, Semiroutine Method for Determining Algal and Sedimentary Chlorophyll Derivatives

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An integrated, quantitative method is described for the determination of chlorophylls and chlorophyll degradation products in freshwater phytoplankton and sediments. Prior to extraction, algal samples were concentrated by filtration through glass fiber filters while sediment samples were lyophilized. All materials were extracted in a mixture of acetone, methanol, and water (80:15:5) by ultrasonication in a glass extraction chamber refrigerated at –10 C. Extracts were filtered through solvent-resistent membrane filters, rather than centrifuged, and then fractionated without prior drying by reverse-phase thin-layer chromatography on oil-impregnated layers of kieselguhr G. Using three chromatographic systems consisting of different developing solvents and oil phases (triolein, parrafin oil, and castor oil), rapid, artefact-free separations of chlorophylls *a*, *b*, and *c* and 18 of their derivatives were obtained. Individual pigments were estimated by direct, in situ thin-layer scanning with a filter fluorometer. The sensitivity and reproducibility of the procedure are ca. 2 ng and 11%, respectively. In routine operation, the method is fast and relatively simple, a complete analysis being accomplished in 1.5 hr.

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Nous décrivons une méthode intégrée et quantitative pour la détermination des chlorophylles et des produits de dégradation de la chlorophylle. Nous avons d'abord concentré les spécimens d'algues par filtration sur fibres de verre, et liophilisé les échantillons de sédiments. Tous les matériaux ont été extraits dans un mélange d'acétone, de méthanol, et d'eau (80:15:5) par les ultrasons dans une chambre d'extraction en verre, réfrigérée à –10 C. Au lieu d'être centrifugés, les extraits ont été filtrés à travers des filtres munis d'une membrane résistante au solvant. Ensuite, et sans dessication préalable, les extraits ont été fractionnés par chromatographie en couche mince et en phase inverse sur des couches de Kieselguhr G imprégnées d'huile. Utilisant trois systèmes chromatographiques consistant en différents solvants de développement et phases huileuses (trioléine, huile de paraffine, et huile de ricin), nous avons obtenu une séparation rapide et libre d'artifices des chlorophylles *a*, *b* et *c*, ainsi que de 18 de leurs dérivés. Nous avons estimé les pigments individuels par balayage direct sur couche mince et in situ à l'aide d'un fluoromètre muni d'un filtre. La sensibilité et la répétabilité du procédé sont approximativement 2 ng et 11% respectivement. Pour une opération de routine, la méthode est rapide et relativement simple, une analyse complète pouvant être faite en 1 heure et demie.

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ALTHOUGH there is now a general recognition that chlorophyll derivatives formed as phytoplankton settle through the water column and accumulate in sediments (Brown et al. 1964; Yentsch 1965b), very little is known for fresh waters about their distribution, quantitative composition, or mechanisms of formation and transformation. Such data are of considerable importance since the chlorophyll derivatives constitute both a potentially useful index of physical and chemical changes in the lacustrine paleoenvironment (Brown 1969) and a source of error in the determination of algal biomass and productivity from chlorophyll estimates (Yentsch 1965a).

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One of the principal obstacles to further studies of these compounds is inadequate analytical methods. Existing indirect procedures for estimating chlorophylls in crude organic extracts are unsuitable. The trichromatic technique of Richards and Thompson (1952) and subsequent modifications of it (Vernon 1960; Vallentyne 1955; Lorenzen 1967) are inherently insensitive and do not distinguish between the individual forms of the native and degraded chlorophylls. Fluorometric techniques (Krey 1958; Yentsch and Menzel 1963; Holm-Hansen et al. 1965; Loftus and Carpenter 1971) are more sensitive than spectrophotometry and permit an estimation of chlorophyll *a*, *b*, and *c* and pheophytin *a*. However, the *b* and *c* pheophytins, the *a* and *b* pheophorbides, and the Mg-containing derivatives (isomers, allomers, and chlorophyllides) are not resolved. Although it is thus clear that direct fractionation procedures for derivative analysis are needed, none are available. Brown (1962) and Koyama et al. (1968) have described paper-chromatographic methods for sediment pigments, but they are too slow and too difficult to quantify for routine work. Both Jeffrey (1968) and Garside and Riley (1969) have reported comprehensive, general-purpose procedures for estimating marine phytoplankton pigments, but the thin-layer chromatography (TLC) methods used in both cases lack the necessary resolution for analyses of chlorophyll derivatives. Furthermore, there exist a number of conflicting reports in the extensive literature on the most suitable procedures for collecting, extracting, and estimating the chlorophylls.

Therefore, in the present study we have undertaken a comparative review of the available techniques and have developed an integrated procedure for the analysis of lacustrine chlorophyll derivatives. Selection was based on the following general criteria: (1) All common derivatives of chlorophylls *a*, *b*, and *c* should be resolved, regardless of source; (2) chlorophyll artefacts must not be formed during collection, extraction, chromatography, and estimation; (3) the sensitivity should be as high as possible, but with high carrying capacity; and (4) the procedure should be as rapid, simple, and as reproducible as possible under a variety of operating conditions.

Materials and Methods

Anacystis nidulans, *Plectonema boryanum*, *Pediastrum boryanum*, *Scenedesmus quadricauda*, *Chlamydomonas reinhardi*, and an unidentified blue-green alga, herein designated BG-Q1, were cultured in the mineral medium of Hughes et al. (1958) as modified by Daley (1971). *Cylindrotheca closterium*, *Nitschia* sp., and *Porphyridium* sp. were grown in the ASP-2 seawater medium of McLachlan (1964) and *Ochromonas* sp. in the medium of Aaronson and Baker (1959) as modified by Daley (1971). Details of culture procedures are given elsewhere (Daley 1971).

Algal cultures and sediments were lyophilized in 10- or 20-ml portions in 50-ml extraction flasks on a Vertis freeze-dryer. For the filtration tests, five 10-ml replicate samples of a dilute suspension of *A. nidulans* were filtered through 2.5-cm disks under a vacuum not exceeding 250 torr. The following filters were used: Whatman glass fiber, no. GFA, coarse, and no. GFC, fine; Reeve Angel glass fiber, ultrafine no. 984H; Gelman Epoxy Versapor membrane, 0.9 μ; Gelman Alpha-6 Metricel membrane, 0.45 μ; Millipore Solvinert UH, 0.5 μ; and MF Millipore HA, 0.45 μ. To overlay filters with $MgCO_3$, 5 ml of a 3% slurry were poured onto the filter and the water was removed by applying vacuum. Approximate filtration rates were measured and the original suspensions and the filtrates were compared in a Klett photoelectric colorimeter (blue filter). All filters were then extracted by ultrasonication.

Solvents used for pigment extraction were stored and prepared as described by Daley et al. (1972). For the ultrasonication tests, a Branson ultrasonic bath and a Sonifyer, model 125, ultrasonic probe with microtip attachment were employed. Stoppered test tubes containing 5 mg of dried BG-Q1 cells in 10 ml of extraction solvent were agitated in the sonic bath for 30 min and the contents then filtered, with washing, through Alpha-6 Metricel filters. The extraction chamber and filtration assembly used in conjunction with the ultrasonic probe are seen in Fig. 1. This unit consisted of a 10-ml glass syringe barrel enclosed in a heavy-walled glass cooling jacket through which methanol, cooled to 15 C, was circulated. A one-way, Luer-lok stopcock connected a micro-syringe filter holder to the syringe barrel above. The assembly was mounted on sliding support bars to permit the microtip to be positioned inside the chamber. Following extraction, the stopcock was opened and the extract was pressure-filtered through an Alpha-6 Metricel filter into a 25-ml volumetric flask. The air line was temporarily sealed into the neck of the extraction chamber by means of a flexible, one-hole plug made of silicone cement.

The chlorophyll derivatives were prepared and chromatographed by reverse-phase thin-layer chromatography (RP-TLC) as described by Daley et al. (1972). For all other TLC methods tested, the authors' original instructions were followed without alteration.

Following chromatography, the pigments were estimated either by elution and spectrophotometry (Daley et al. 1972) or directly on the chromatogram by reflectance fluorometry. For the latter, a Turner filter fluorometer equipped with a far-red sensitive photomultiplier (no. R-136, Hitachi TV Corp, N.Y.) and a Turner Model II TLC scanner door was used. The primary and secondary filters were, respectively, a Kodak wratten, narrow pass filter, no. 47B, and a Corning sharp-cut filter, no. 2–64. Chromatograms were scanned at a rate of 1 cm/min with a permanently fixed slit width of 1 mm. A 25-cm strip-chart recorder was connected to the fluorometer through a calibrated range expander (the schematic diagram for this is given in Fig. 2). Peak areas

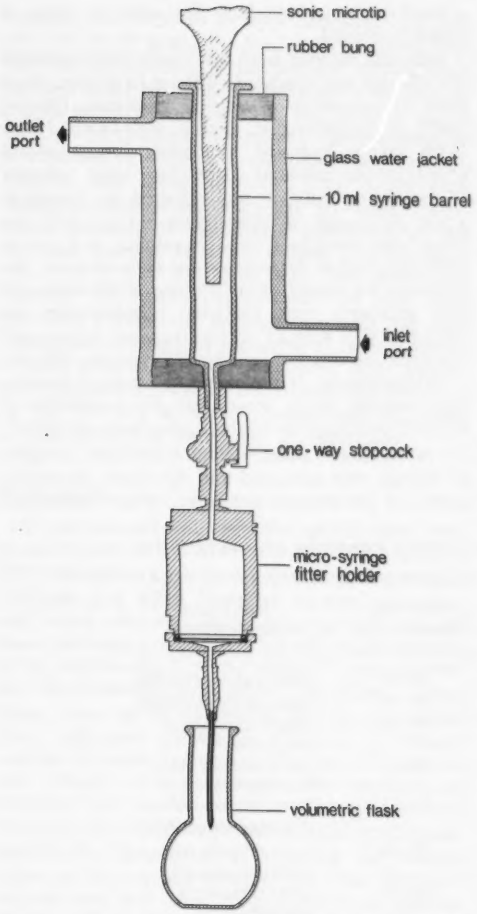

FIG. 1. Schematic diagram of the extraction and filtration unit used in conjunction with the ultrasonic probe.

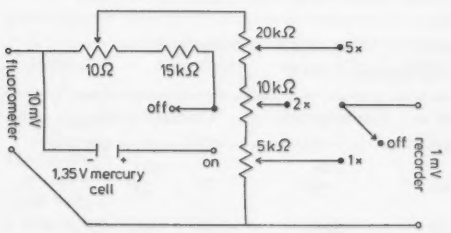

FIG. 2. Schematic diagram of the calibrated range expander for the fluorometer.

were determined automatically with a mechanical integrator.

The scanner was calibrated by spotting known concentrations of each pigment on RP-TLC plates. The areas of the pigment peaks thus obtained were then plotted against the amount of pigment applied. Spectrophotometric estimates were based on the extinction coefficients reported by Brown (1968). Extinction data were unavailable for several of the isomeric and allomeric *a* and *b* chlorophyll derivatives. For these, the coefficients of the parent compounds were used (Daley 1971). Chlorophyll *c*, pheophytin *c*, and bacteriochlorophyll *a* were calculated from the coefficients of Strain and Svec (1966).

Results and Discussion

The complete procedure involves five interrelated stages: sample acquisition, pigment extraction, drying and concentration of extracts, fractionation, and quantitative estimation. For simplicity in presentation, the development of each step will be considered separately.

Sample Acquisition

Phytoplankton have usually been concentrated by filtration rather than centrifugation. However, the commonly used cellulose triacetate membrane filters (e.g. Millipore MF) that dissolve during extraction are unsuitable for use with direct fractionation methods as they interfere with subsequent chromatographic separations. Therefore, a variety of solvent-resistent membrane filters and glass fiber depth filters were compared as to filtration rate, retention efficiency, and ease of pigment extraction (Table 1). None of the membrane filters were found suitable for chlorophyll derivative analysis. With the Alpha-Metricel and the Solvinert types, the filtration rates were impractically low, while extraction of the chlorophylls off the Versapor discs was incomplete. On the other hand, all the glass fiber filters tested exhibited acceptable flow rates and extraction characteristics, and only one (GFA) failed to provide quantitative retention.

These latter results differ from those obtained by Humphrey and Wooton (1966) and Garside and Riley (1969) for marine algae. They reported that adequate retention with glass fiber filters could be obtained only by prefiltration with fine $MgCO_3$ powder. In the present tests with *A. nidulans*, a similar enhancement of retention efficiency with $MgCO_3$ was not observed (Table 1). Moreover, in preliminary work with samples containing pheophorbides or chlorophyllides, it was often observed that less pigment was extracted from filters overlain with $MgCO_3$ than from the same filter used alone. Subsequent tests with pure derivatives showed

that the nonphytylated chlorophyllides and pheophorbides were strongly adsorbed onto the $MgCO_3$ particles during extraction and thus were subsequently lost from the extract upon filtration (Table 2). In some instances, the losses of chlorophyllide *a* and pheophorbide *a* were as high as 70 and 20%, respectively; the glass fiber itself showed no adsorption.

To further evaluate the retention characteristics of the glass fiber filters, an additional 8 species of algae, 2 deep-water phytoplankton samples, and a broken-cell preparation of BG-Q1 cells were tested. In all cases, the retention efficiency, determined colorimetrically, was 95% or higher. Hence, it would appear that the potential error from adsorption by $MgCO_3$ when it is used during filtration is greater than the error from incomplete retention when it is not used. Whatman GF/C filters without $MgCO_3$ were therefore used in all subsequent work. A more suitable filter for chlorophyll derivative studies, namely, a sturdy, solvent-resistent, easily-extracted membrane filter with a small pore size and high flow rates in water, is urgently needed.

Sediment samples have previously been extracted wet (Sanger and Gorham 1970) or have been dried prior to extraction by vacuum desiccation (Brown 1968) or lyophilization (Kemp and Lewis 1968). With highly hydrated sediments, drying would appear to be essential. Otherwise, large volumes of extracting solvent are required to maintain a low percentage of water in the extract, with the result that additional, time-consuming, and potentially destructive procedures for concentrating the pigments are needed. Furthermore, when some wet clay sediments were extracted ultrasonically, an emulsion was formed which rendered subsequent filtration and chromatography of the extract difficult.

Lyophilization is preferable to vacuum desiccation because of its speed, but the possibility of artefact formation has not been adequately evaluated. To do so, fresh (filtered) and lyophilized samples of BG-Q1 were extracted and thin-layer chromatograms of the extracts compared. When lyophilized

TABLE 1. The filtration characteristics of various membrane and depth filters.

Filter	Relative filtration rate (%)[a]	Retention efficiency (%)	Extraction characteristics
Alpha-6 Metricel	15	99±4	inert, easily extracted
Solvinert, UH	25	101±6	inert, easily extracted
Versapor	70	97±5	invert, incomplete extraction
Reeve Angel, 984H	110	98±3	inert, releases cells, disintegrates easily
GF/A	180	81±5	inert, releases cells, disintegrates easily
GF/C	100	96±4	inert, releases cells, disintegrates easily
GF/C + $MgCO_3$	90	102±8	polar derivatives adsorbed.

[a]Compared to rate of GF/C filter (ca. 120 ml/min). Values only approximate.

TABLE 2. Recovery of chlorophyll *a* and chlorophyll *a* derivatives following ultrasonication with glass-fiber filters and magnesium carbonate.

Treatment	% recovery[a]			
	Chlorophyll *a*	Pheophytin *a*	Pheophorbide *a*	Chlorophyllide *a*
GF/C filter	93	99	96	102
$MgCO_3$	97	98	79	29
GF/C filter + $MgCO_3$	101	94	83	21

[a]Two replicates for each treatment.

samples were exposed to light intensities in excess of *ca.* 4×10^4 ergs cm^{-1} sec^{-1}, serious chlorophyll destruction occurred. In samples that melted while drying, either from accidental loss of vacuum or excess salt concentrations in the sample, up to 30% of the chlorophyll *a* present was converted to allomerized or isomerized chlorophyll *a*. However, samples that remained frozen and in the dark during lyophilization contained the same (negligible) amounts of chlorophyll derivatives as did the fresh samples. To date, 14 algae, 3 phytoplankton, and 8 lake sediments have been lyophilized without chlorophyll degradation. On the basis of these results, all sediment samples for this study were prepared for extraction by lyophilization.

Tests showed that, if necessary, both filtered and lyophilized materials could be stored at –20 C in the dark for at least 2 months without losses of chlorophyll or formation of derivatives.

Extraction

In previous studies, the chlorophylls have been extracted with such solvent mixtures as 90% acetone, 90% methanol, mixtures of acetone and methanol (Garside and Riley 1969), or acetone, methanol, and water (Brown 1969). Comparative tests of these extractants indicated that the latter mixture, in the proportions 80:15:5 ("80/15/5"), was superior for the lacustrine algae tested. In general, it was found that the water content should not exceed 10%, otherwise extraction efficiency is reduced and the possibility of enzymic oxidation or hydrolysis (Strain 1954) enhanced. The addition of methanol was found to facilitate the extraction of the polar chlorophyll derivatives but did not promote pheophytin formation, as reported for marine algae by Garside and Riley (1969). The "80/15/5" mixture has also been recommended for the extraction of algal carotenoids (Beaulieu 1968).

Several procedures for increasing extraction efficiency have been reported. These include overnight shaking (Creitz and Richards 1955), mechanical grinding (Humphrey and Wooton 1966), and disruption in the ultrasonic bath (Nelson 1960; Garside and Riley 1969). Some confusion exists as to which procedure is preferable. Overnight extraction with shaking is reported to be incomplete (Humphrey and Wooton 1966). Mechanical grinding, although more efficient than overnight digestion, has been shown to cause pigment degradation (Kerr and Subba Rao 1966). Garside and Riley (1969) found that agitation in an ultrasonic bath provided rapid complete extractions without degradation, but Yentsch and Menzel (1963) obtained the opposite results. As most of the existing data has come from marine studies, a reevaluation of these common extracting procedures using freshwater algae was undertaken. Such comparative tests are complicated by the fact that extraction efficiency varies with cell concentration, chlorophyll content, type of extracting solvent, and species of algae. Nevertheless, it was clear that overnight shaking, grinding, and sonic bath disruption were all unsuitable for the present purposes. In most tests (Table 3) incomplete recoveries or chlorophyll degradation or both were found. For these reasons, the use of the more powerful sonic probe was evaluated. As this instrument generally provided better pigment recoveries in less time and with less degradation than the other tested methods (Table 3), a systematic determination of the minimum requirements for its use, in combination with the extractor-filtration unit (Fig. 1), was made.

A variety of precautionary procedures have been recommended to prevent the formation of artefacts during extraction. These include operation at low temperatures to minimize isomerization; the addition of antoxidants such as N_2, H_2S, as-

Table 3. A comparison of different chlorophyll extraction procedures. Three 5-mg samples of lyophilized BG-Q1 cells were analyzed with each technique.

Method	Extraction time (*min*)	Extraction temp (*C*)	Extraction efficiency (%)	Artefact content (% *of total*)	Artefacts formed
Glass beads + shaking	720	25	35±12	15	chlorophyll *a'* allom. chlorophyll *a*
Mechanical grinding	20	25	ca. 80	ca. 5	chlorophyll *a'*
Sonic bath disruption	30	25–50	60±11	6	chlorophyll *a'*
Sonic probe disruption	5	0	100±3	trace	

corbate (Kim 1966), or ethoxyquin (Nelson and Livingston 1967) to reduce chemical and enzymic oxidation; and the use of basic substances such as $MgCO_3$ or dimethylaniline (Vallentyne 1955) to prevent the formation of pheo-pigments. When extracts were sonicated without cooling, the temperature quickly rose to *ca.* 40 C in the chamber and small amounts of chlorophyll isomers were formed. If the extraction time exceeded 25 min, the same isomers were formed, even at 0 C. In addition, some pigment was lost from the open chamber as a result of sonic aerosoling. However, only trace amounts ($< 1\%$) of isomers were formed with short extraction times and low temperatures. With all species tested, neither antoxidants nor antacids were essential to prevent pigment degradation, as a result, perhaps, of the short extraction times. However, their precautionary use in routine analysis is essential, as enzyme activities and organic acid levels may vary from species to species. Of the antoxidants, ascorbic acid was the most suitable. N_2 outgassing and H_2S additions are troublesome, and ethoxyquin, being fluorescent, interferes with the visual inspection of chromatograms under UV light. Either $MgCO_3$-saturated water (free of solids) or strong phosphate buffer in place of the water in the extracting mixture was found most suitable for maintaining basic conditions in the extract. Chlorophyllides are reported to be formed by the action of chlorophyllases released during extraction in higher plants (Strain 1954) and marine algae (Barret and Jeffrey 1964). Such postmortem alterations are best prevented by the use of extraction mixtures low in water, such as "80/15/5", and by the removal of the cell debris from the pigment extract as quickly as possible. For the latter, direct filtration as used here is faster, simpler in operation, and more easily quantified than centrifugation.

For operational simplicity, we first attempted to select a single power setting and insonation time for the sonic probe that would provide quantitative recovery of the chlorophylls regardless of their source, but without success. It was found (Table 4) that, for a given power setting, the extraction time varied with the quantity of cells being extracted and, for a given quantity of cells, with the pigment source and with the age, physical and physiological condition, genus, and even strain of alga. Furthermore, the insonation efficiency of the probe appeared to change over a period of several months. With experience, operating conditions that provided complete extraction could usually, but not always, be chosen. Consequently, it was necessary to determine or recheck extraction efficiencies with widely differing types of samples. The common recommendation to extract until the cell debris is colorless is an inadequate criterion for extraction, as phytoplankton debris from fresh waters is usually blue-grey to brown in color. Hence, the filters containing the debris from the first extraction were resonicated to determine if extraction was complete. If doubt still remains, the cell remains can be extracted overnight in 12-N HCl and examined spectrophotometrically for the presence of pheophorbide.

TABLE 4. Minimum times for complete chlorophyll extraction from a variety of sources using the ultrasonic probe. Extraction conditions: 6 ml "80/15/5," Branson generator power level 4, lyophilized samples.

Sample type	Quantity (*mg*)	Extraction time[a] (*min*)
Freshwater		
BG-Q1, 10 hr	5	3.5
BG-Q1, 10 hr	10	5.5
BG-Q1, 10 hr	20	13
BG-Q1, 90 hr	5	3.0
BG-Q1, 190 hr	5	2.0
A. nidulans	5	6.0
P. boryanum	5	4.0
S. quadricauda	5	18
S. quadricauda + glass beads	5	8.0
S. quadricauda[b]	5	7.0
Ochromonas sp.	5	15
D. pulex feces	5	27
D. pulex feces + glass beads	5	11
BG-Q1, broken cell preparation	5	1.5
Lake Ontario sediment	300	3.0
Marine		
C. Closterium	5	<0.5
Nitschia sp.	5	<0.5
Porphyridium sp.	5	<0.5

[a]Two extraction curves obtained for each sample.
[b]Cambridge culture collection, no. 276/4C

Such wide variations in extractability with lacustrine pigment samples poses problems regardless of the extraction procedure employed. Ultrasonic disruption has the advantage that the necessary tests can be done quickly. In general, the ease of extraction of a given amount of pigment increased with source according to the series: detrital and fecal material < lacustrine algae < freshwater sediments < marine algae. These observations may explain in part why the common procedures used in marine analyses, such as sonic bath extraction and mechanical grinding, were ineffectual for freshwater species. The extraction of difficult samples (e.g. zooplankton feces) was facilitated by the addition of 50-μ glass beads to the extraction

chamber (Table 4), rather than by the use of higher power settings on the sonicator. With the latter, foaming can occur with a consequent decline in power output.

The possibility that pigment losses were occurring during extraction and filtration was assessed as follows. A known amount of chlorophyll *a* was sonicated in "80/15/5" for 5 min, filtered with washing into a volumetric flask, and remeasured spectrophotometrically. The average recovery for 10 replicate samples was 98 ± 2%. Reproducability was assessed by extracting 5-mg (dry weight) samples of BG-Q1, filtering the extracts, and comparing their adsorbances. The standard deviation of 10 replicates was 3.9%. In routine use, the complete procedure has permitted rapid, artefact-free, quantitative extractions of 12 algal, 6 phytoplankton, and 6 sediment samples.

Drying and Concentration of Extracts

As most adsorption TLC methods require developing solvents of low polarity, it has previously been necessary to dry the pigment extract prior to chromatography (Strain and Svec 1969). This has been accomplished in several ways: phase transfer to nonpolar solvents (Jeffrey 1968), vacuum aspiration (Brown 1968), and rotary flash evaporation at elevated temperatures (Garside and Riley 1969). However, Brown (1969) has cautioned that chlorophyll artefacts, especially allomers, may be formed as a result of such techniques. Comparative tests were therefore made. Solutions of chlorophyll *a* in "80/15/5" were reduced to dryness by vacuum aspiration, evaporation under a stream of nitrogen, and rotary flash evaporation at 50 C, then redissolved in diethyl ether. After standing for 1 hr, chromatograms of these solutions and of undried controls were compared by fluorescent scanning. Small amounts of derivatives were produced by all three procedures (Table 5). In contrast to the results of Garside and Riley (1969) for marine algae, rotary evaporation, although fastest, produced the greatest number and the largest amounts of derivatives. In the present study, the need for drying has been eliminated. Wet extracts (in "80/15/5") may be applied directly to the reverse-phase thin-layers chosen for pigment fractionation (see below and Daley et al. 1972). When necessary, extracts were concentrated by partial evaporation under a stream of nitrogen. As long as the volume reduction did not exceed 50%, no derivatives were formed.

Pigment extracts in "80/15/5" were found to undergo very serious degradation in the first week of storage, even at –15 C in the dark. Therefore, all extracts were chromatographed and analyzed immediately following extraction. Storage, if necessary, should not exceed 12 hr.

Thin-Layer Chromatography.

A reverse-phase, thin-layer chromatography system (RP-TLC) designed specifically for the analysis of chlorophyll derivatives has been reported elsewhere (Daley et al. 1972). The method employs three different thin-layer systems consisting of different development solvents and impregnation oils: a "low-range" (LR) system for resolving pheophytins, a "mid-range" (MR) system for chlorophylls, and a "high-range" (HR) system for pheophorbides and chlorophyllides. Its suitability for present purposes was compared with a number of other adsorption and partition TLC methods employing layers of sugar (Colman and Vishniac 1964; Madgwick 1965; Smith et al. 1965; Jeffrey 1968), cellulose

Table 5. The effect of different drying procedures on the formation of derivative artefacts in chlorophyll *a* solutions.

Drying procedure	Reduction time (*min*)	Derivative content (% *of total*)[a]	Derivatives formed
Control in "80/15/5"	0	trace	
Evaporation with N_2	60	1.9	allomerized chlorophyll *a*
Vacuum aspiration	45	2.8	allomerized chlorophyll *a*
Rotary flash evaporation	15	9.0	allomerized chlorophyll *a* chlorophyll *a'*

[a]Mean of three determinations.

(Bacon 1965; Schneider 1966), silica gel (Grob et al. 1961; Lynn Co and Schanderl 1967; Riley and Wilson 1965), and kieselguhr (Egger 1962; Kim 1966).

The selectivity of these procedures, used either singly or in combination, were found to be inferior to the RP-TLC method. With it, chlorophylls *a*, *b*, and *c*, 18 identified derivatives and 8 unidentified derivatives of chlorophylls *a*, *b*, and *c*, bacteriochlorophyll *a*, and 7 derivatives of bacteriochlorophyll *a* were resolved.

A number of additional advantages of RP-TLC were noted. The pigment zones are sharp and uniform and double tailing effects do not occur. General reproducibility is high due to the relative insensitivity of the layers to humidity changes and to variations in the number of pigments in a sample. Because the solvents and extracts contain water, the need for drying procedures that may cause degradation is eliminated. Artefacts are not formed during spotting or development and pigments are stabilized within the oil-impregnated layers. Carrying capacity is moderately high and developments are fast. For these reasons RP-TLC was chosen for the present study.

Quantitative Estimation

Pigment estimations following chromatography have usually been accomplished by elution and spectrophotometry. Although reproducible measurements can be obtained in this manner with RP-TLC, recovery efficiencies were relatively low (Daley et al. 1972) and the technique is slow and tedious. Garside and Riley (1969) have reported the use of a reflectance photometric scanner that permits the estimation of carotenoids and chlorophylls directly on the chromatogram. As the sensitivity of their procedure was moderately low for the chlorophylls, we have evaluated the use of the direct scanning Turner fluorometer (Jänchen and Pataki 1968) for in situ estimation. A similar procedure has recently been published for porphyrins (Doss 1970).

Traces of two typical chromatogram scans are seen in Fig. 3. With RP-thin-layers, pigment peaks are usually symmetrical. The instrument is fast and simple to operate, estimation of a full chromatogram being accomplished in 20 min. The maximum sensitivity of the instrument for chlorophyll *a* was ca. 1 ng/spot, a 100-fold increase over conventional spectrophotometry. Optimum scanner resolution and sensitivity was obtained with a 1-mm slit width and a scanner speed of 1 cm/min.

Calibration curves (Fig. 4) for all derivatives tested were found to be linear up to ca. 0.3 µg pigment. Representative calibration coefficients, expressed as arbitrary integrator units/µg pigment, are seen in Table 6. For any given pigment, the absolute value of the coefficient varied with a number of factors: layer thickness, kind and concentration of impregnating oil, impregnation procedure (dipping vs. development), plate dryness, fluorometer slit width, and distance of development. With the last factor, the variation was the result of changes in pigment band width, wider bands resulting in higher calibration coefficients. Recalibration was necessary whenever any of the above factors were changed. Holm-Hansen et al. (1965) and, more recently, Loftus and Carpenter (1971) have detailed several important precautions concerning calibration of the Turner fluorometer. In addition to these, it is essential that the slit width on the TLC-scanner door not be altered, once chosen, as the adjustment mechanism and scale are not reproducible. The total variation of the in situ estimation technique with the present standardized procedure is slightly inferior to conven-

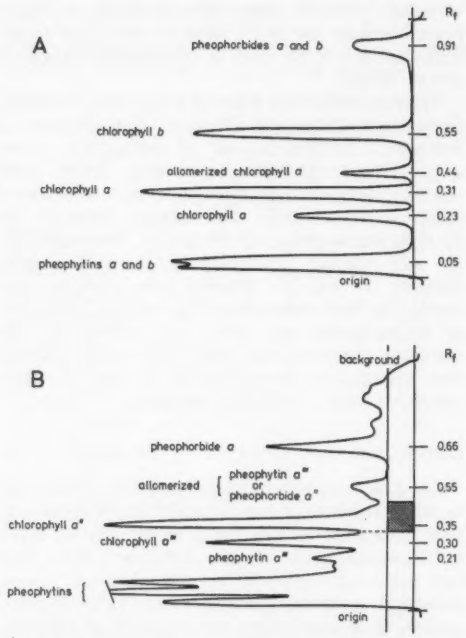

FIG. 3. Representative recorder traces of MR thin-layer chromatograms: A, Extract of *Scenedesmus quadricauda* cells and fecal material from *Daphnia pulex* fed on *S. quadricauda* cells. Solution stored in a dark place at 10 C for 2 months; B, Extract of surficial sediment from Lake Opinicon, Ont. The background area to be subtracted from the chlorophyll *a''* peak is shown by the shaded area.

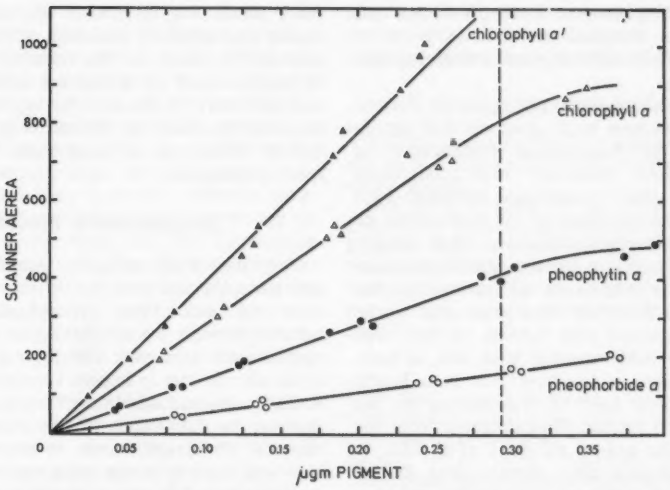

FIG. 4. Representative calibration curves for the determination of the chlorophyll derivatives by fluorescence scanning. Dashed line indicates upper limit of linearity. Instrument settings: fluorometer range selector, 10×; external range expander, 1×; slit width, 1 mm.

TABLE 6. Specific calibration coefficients for chlorophyl derivatives determined with the Turner 111 filter fluorometer and TLC scanner door.[a]

Pigment	Chromatographic system[b]	Molecular weight	Calibration coefficient[c]
Chlorophyll *a*	MR	893.5	2817
Chlorophyll *a'*	MR	893.5	3802
Allomerized chlorophyll *a*	MR	925.5	2817
Pheophytin *a*	1.5% Triolein	871.2	1418
Allomerized pheophytin *a*	1.5% Triolein	903.2	751
Pheophorbide *a*	12% Triolein	592.7	516
Allomerized pheophorbide *a*	12% Triolein	624.7	480
Chlorophyl *b*	MR	907.5	2041
Chlorophyll *b'*	MR	907.5	4808
Allomerized chlorophyll *b*	MR	939.5	3378
Pheophytin *b*	1.5% Triolein	885.2	3145
Allomerized pheophytin *b*	1.5% Triolein	917.2	2510
Pheophorbide *b*	12% Triolein	638.6	2410
Allomerized pheophorbide *b*	12% Triolein	670.7	1674
Chlorophyll *c*	MR	510.7	605
Pheophytin *c*	MR	486.4	ca. 900
Bacteriochlorophyll *a*	MR	911.5	270

[a]Instrument settings: fluorometer range selector 10×; external range expander, 1×; slit width, 1 mm.

[b]See Daley et al. (1972) for details.

[c]The slope of the "least square" regression line obtained from at least 20 determinations at different pigment concentrations.

tional elution and spectrophotometry. With a single chromatogram, a standard deviation ($n = 8$) of 4.1% was obtained; with different chromatograms ($n = 12$), 9.9%.

In some lake sediment extracts, unknown fluorescent contaminants have been observed that significantly increase the background fluorescence of the pigment scans. Although these compounds fluoresced blue, they transmitted sufficient light through the secondary filter of the fluorometer to interfere with chlorophyll estimations. Their identity is not known. Correction for this interfering background was done graphically by subtracting the background area from the total peak area of the scan. The background was defined as that area bounded by the 0-fluorescence base line, a horizontal line parallel to the base line at a height equal to the lowest level of fluorescence on the scan and the two vertical lines dropped from the beginning and the end of the peak (Fig. 3B).

These contaminants also absorb light in the 670 mμ region of maximum chlorin fluorescence; visible spectra of chromatographic eluates showed gradually decreasing absorbances from 380 to 800 mμ. As a result, the corrected fluorometric scans tend to underestimate the amount of chlorophylls present in a sample. In experiments where known amounts of chlorophyll *a* were mixed with lake sediment extracts and chromatographed, the corrected peak areas for chlorophyll *a* were reduced by 3–6% in comparison to the peak areas of control extracts containing the same amounts of chlorophyll *a* chromatographed alone. Since the amount of contaminants in each sample may not be constant per unit of pigment, these effects must be investigated in each sedimentary environment sampled.

The high sensitivity of the fluorometer, together with the permanent record it provides, eliminates the risk of missing minor pigment components (e.g. chlorophyll *b* or *c* derivatives), which can occur when inspecting chromatograms with a long-wave UV hand lamp. Individual pigments cannot, of course, be identified directly from the scanner trace, but must be obtained from R_f or R_p values (Garside and Riley 1969; pheophytin *a*, chlorophyll *a*, and pheophorbide *a* are used as reference pigments on the LR, MR, and HR thin-layers, respectively), cochromatography with authentic reference materials, or elution and spectrophotometry. For the latter purpose, sufficient pigment was obtained by development of separate, streaked chromatograms.

The variation of the complete analytical procedure was determined by extracting, chromatographing, and scanning two replicate (5-mg) samples of lyophilized BG-Q1 cells each day for 5 days, using plates impregnated 1 day before the tests began. The standard deviation of the 10 replicates was 10.9%. Most of this variation would appear to be the result of differences in the batch, age, and thickness of the thin-layers. Attempts to reduce this variation by the use of preformed, commercial thin-layers of kieselguhr have thus far been unsuccessful.

Recommended Procedure

Lyophilize fresh sediment samples in the dark and store the dry powders in sealed vials at –20 C in the dark. Filter phytoplankton and algal cultures through 5.5-cm GFC glass filters under a vacuum not exceeding 250 torr. Add the sample to the filter holder in known volumes until the filter is darkly colored and the filtration rate begins to decrease (*ca.* 0.3–3 liters of lake water, 5–200 ml of culture). While still moist, remove and trim the filter and store in sealed plastic petri dishes in the dark at –20 C. Do not use $MgCO_3$ for filtration and do not desiccate or steam treat the filters.

Extract and analyze all materials within 2 months of storage. Place the frozen filter or a weighed amount of sediment (usually 100-500 mg) in the extraction chamber with ca. 0.1-gm ascorbic acid and 6 ml of "80/15/5" (80% acetone, 15% methanol, and 5% distilled water saturated with $MgCO_3$ but free of particles). Position the microtip of the ultrasonic probe 1 cm from the bottom of the extraction chamber and sonicate at an appropriate power density for a period of time that is known or expected to give complete extraction (with the Branson probe, 10 min at power level 4 is usually adequate). Lower the extractor assembly and rinse the probe tip free of adhering material. Open the one-way valve beneath the chamber and filter the extract through a 2.5-cm Alpha-6 Metricel filter into a 25-ml volumetric flask. Rinse the chamber with eight 2-ml portions of "80/15/5." When the flask contents have warmed to room temperature, fill the volumetric to the mark and cap. Carefully remove the filter from the holder and resonicate in "80/15/5" as described above. Compare the first and second extracts colorimetrically. If the absorbance of the second extract is less than 2% of the first, extraction is considered complete. If not, discard the extracts and sonicate a second sample for a longer time or in the presence of 50-μ glass beads. When necessary, store the extracts at –20 C in the dark for no longer than 12 hr.

Place an MR thin-layer in the spotting box, apply an appropriate volume (100-300 μliter) of extract onto the unimpregnated portion of the layer, and develop it in an S-chamber according to the procedure of Daley et al. (1972). Two replicate

pigment spots per chromatogram, ca. 1 cm wide and 6 cm apart, are preferred. Following development, mark the location of the origin, front, and pigment spots and dry the layer for 10 min by placing it upright 30 cm from an electric fan. Position the plate in the plate holder of the fluorometer so that the rows of pigment spots are parallel to the axis of scan. Manually align the chromatogram in front of the light slit. With the external range selector positioned on the 1× setting, choose an appropriate sensitivity range on the fluorometer (either 10× or 30×), using the pigment present in highest quantity. Scan the chromatogram. For accurate determination of pigments with peaks less than 10% of full scale deflection on the recorder, repeat the scan using the 5× setting of the external range expander.

If pheophytins, pheophorbides, or chlorophyllides are present on the MR chromatogram, analyze additional portions of the extracts on the LR and HR systems using the above procedure. Note the integrator reading, R_f or R_p values, and color for each pigment. Calculate pigment amounts from the equation:

$$\mu\text{moles pigment} = \frac{A \cdot V}{R \cdot C \cdot v \cdot M}$$

where A is the peak area in arbitrary units, C is the calibration coefficient, M is the molecular weight, R is the total amplification of the scan relative to the lowest sensitivity (10 × internal expansion, 1 × external expansion), and V and v are the total extract volume and chromatogram spot volume, respectively, in milliliters. If the identity of any pigment is in doubt, cochromatograph the extract with authentic reference compounds or obtain visible spectra of the pigment. Correct for background fluorescence, if present in the sample, by calculating the area of the rectangle subtended by vertical lines dropped from the beginning and end of the peak and the horizontal line drawn from the lowest point on the trace to cross the two vertical lines.

Conclusions

The greatest single drawback in the past to the use of direct fractionation procedures for chlorophyll analyses has been the slowness of the technique. To perform a single analysis by elution and spectrophotometry 4–6 hr were normally required (Gray and Kemp 1970; Daley et al. 1972). With the present procedure, the analytical time has been reduced considerably by the use of ultrasonic extraction, direct filtration of extracts, in situ scanning, and direct chromatography of crude extracts without prior drying or concentration. A single analysis can be completed in 1.5 hr.

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Diet, Time and Place of Spawning, and Environments Occupied by Creek Chub (*Semotilus atromaculatus*) in the Mink River, Manitoba

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MOSHENKO, R. W., AND J. H. GEE. 1973. Diet, time and place of spawning, and environments occupied by creek chub (*Semotilus atromaculatus*) in the Mink River, Manitoba. J. Fish. Res. Board Can. 30: 357–362.

Creek chub (*Semotilus atromaculatus*) are omnivorous with a diet consisting of berries, insects, crayfish, noncyprinid fishes, and frogs. In 1971, in the Mink River, most spawning occurred between the middle and end of May in shallow (< 25 cm) fast-flowing (> 15 cm/sec) waters. Fecundity varied from 1146 to 7539 ova per female. In summer most chub in their 1st and 2nd years occupied moderately deep waters (< 50 cm) with little current (0–30 cm/sec), while most older chub occupied deeper waters. In autumn chub were most abundant in deep (> 50 cm) sheltered pools, where they overwintered.

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Le mulet du Nord (*Semotilus atromaculatus*) est un poisson omnivore dont le régime est constitué de baies, insectes, écrevisses, poissons autres que les cyrpinidés, et grenouilles. En 1971, la fraie a eu lieu surtout du milieu à la fin de mai, dans des eaux peu profondes (< 25 cm) à courant rapide (> 15 cm/sec). Le nombre d'oeufs varie de 1146 à 7539. En été, la plupart des mulets du Nord dans leurs première et deuxième années occupent des eaux modérément profondes (< 50 cm) à faible courant (0–30 cm/sec), alors que la plupart des mulets plus âgés habitent des eaux plus profondes. En automne, les mulets sont le plus abondants dans les fosses profondes (> 50 cm) abritées, où ils hivernent.

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THE creek chub (*Semotilus atromaculatus*) is a large cyprinid and, although common and widely distributed in streams and rivers across central and eastern North America, information is scant on most aspects of its life history. Accounts are incomplete on diet (Barber and Minckley 1971) and on environments occupied (Eschmeyer and Clark 1939; Minckley 1956), while age and growth (Greeley 1930; Dinsmore 1962) and reproductive behavior (Reighard 1910; Miller 1964, 1967; Sisk 1966) have been described. This note provides information on diet, time and place of spawning, fecundity, and abundance of creek chub gathered from May 1970 to August 1971 in different environments in the Mink River.

Materials and Methods

The Mink River was divided into upper, middle, and lower zones, as follows:

	Zone		
	Upper	Middle	Lower
Length (*km*)	8	17	22
Mean gradient (*m/km*)	2	7	3
Overhanging vegetation	Dense willow; tall grasses	Dense, high stands of willow, poplar	Very sparse willow, poplar
Substrate[1]	Mostly silt	Variable, sand–rubble	Variable, clay–gravel

[1]Particle sizes as in Lagler (1956).

The meandering upper zone contains beaver dams while alternating pools and riffles occur in the middle zone. A marsh 2 km long separates the middle and lower zones. The latter is primarily a man-made, straight, high-diked ditch (See Moshenko 1972 for further details).

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To estimate their density in each zone, chub were collected monthly (May-October) at several sites. A minimum total of 325 m² was sampled in each zone. Furthermore, seven environments were defined (Table 1) and densities of chub by size-group in each were estimated monthly for the middle zone only. Four size-groups were defined as follows:

	Size-group			
	1	2	3	4
Fork length (mm)	<50	50–80	81–120	>120
Age of males	0	1	2–3	4–7
Age of females	0	1	2–4	5–6

Size-group 1 could be separated from others by length. Larger fish were aged by the scale method. The ranges of ages of fish in some of the length groups are approximate in that, especially in groups 3 and 4, several fish were older or younger than indicated in the table (See Moshenko 1972 for further details).

Prior to making a collection in any environment, the area to be sampled was enclosed with barrier nets (3.5-mm diam mesh), and all chub were removed using a two-man seine (1.4 mm × 2.0 m; 3.5-mm diam mesh) or a pulse electrofisher (Brookmaster MK10, d-c 12 v, 8 amp) or both. Chub were anesthetized (tricaine methanesulphonate), measured to the nearest mm fork length, sexed (2 year and older), and placed into recovery tanks before being returned to the stream.

To determine places of overwintering of size-groups 3 and 4, gillnets (18–60 mm mesh size) were set under the ice in deeper environments (middle zone) on December 5–8. The area of net fished (m²) for 24 hr was 20 in moderately deep pools and in slow deep channels, 48 in deep pools, and 96 in sheltered deep pools.

For analysis of diet, each month some chub were preserved in 10% formalin and later transferred to 40% isopropyl alcohol. Contents of the anterior portion of the intestinal tract were identified at least to family and weighed (± .001 g), and numbers of each food item were recorded.

Spawning period was indicated by nest-building activities of male chub and indices of maturity (total gonad weight/body weight (± .001 g) × 100). Indices were obtained from chub collected weekly from April 28 to June 9, 1971. To determine place of spawning, estimates were made of both density of nest sites and adult chub by sex in each environment in the middle zone. Fecundity was determined from 40 chub collected just prior to spawning. Ovaries were weighed (± .001 g) and the total number of ova per female was calculated by proportion using the mean weight of two samples of 100 randomly mixed eggs taken from each female.

To estimate changes in environments occupied between seasons, and distances and directions moved, movements of chub of size-groups 2–4 were examined in the upper zone. Chub were captured, anesthetized, marked with either finclips or a hyfricator (Owens and Gebhardt 1968), and released at site of capture after recovery.

Results

Diet

Size-group 1 consumed aquatic and terrestrial insects (Fig. 1). Size-groups 2–4 ate mostly aquatic insect larvae in May and June, and brook stickleback, crayfish, and plant material (berries of *Cornus*, *Prunus*, and *Viburnum*) in July and August. In September and October size-groups 2 and 3 consumed mainly aquatic insect larvae, crayfish, brook stickleback, and Johnny darters, while size-group 4 ate mostly frogs.

All fishes eaten by chub were noncyprinids except that one had consumed several fry of northern pearl dace. Yet brassy minnow, northern pearl

Table 1. Characteristics of environments studied.

	Fast shallow channels	Slow shallow channels	Shallow pools	Moderately deep pools	Slow deep channels	Deep pools	Sheltered deep pools
Surface velocity (*cm/sec*)[a]	>30	15–30	0–15	0–15	15–30	0–15	0–15
Water depth (cm)[b]	<25	<25	<25	25–50	>25	>50	>50
Substrate[c]	gravel–coarse rubble	gravel–fine rubble	silt–gravel		sand, gravel, rubble	silt–gravel	
Water surface with cover (%)[d]	0	0	<5	<5	<5	5–10	>10

[a]Measured by timing a floating object.
[a,b]Except during freshets.
[c]As defined by Lagler (1956).
[d]Overhanging bank, overhanging terrestrial and emergent aquatic vegetation, fallen overhanging or submerged trees, and boulders.

dace, and common shiner are present in environments occupied by chub and are more numerous than chub, stickleback, or Johnny darter (G. E. Newsome personal communication). In addition, blacknose dace and fathead minnow are also abundant in places with chub. A pheromone alarm substance is present in the epidermis of many cyprinids and work using skin extracts has shown that strong intraspecific and interspecific fright reactions occur amongst minnows (Pfeiffer 1962, 1963; Reed 1971). Thus, if a chub were to attempt to eat cyprinid, it may itself become frightened. This, combined with the evasive action taken by other potential cyprinid prey, would result in very few cyprinids consumed by chub. However, some populations of chub do consume cyprinids such as common shiner and blacknose dace, and are even cannibalistic (Greeley 1930; Minckley 1963; Noble 1965; Barber and Minckley 1971). Consumption of cyprinids by chub may occur when other suitable food is in short supply.

Time and Place of Spawning and Fecundity

Males were observed constructing and defending nest sites from May 14 to 28, when daytime water temperatures exceeded 14 C (Fig. 2A). This activity ceased temporarily on May 17–18, when daytime temperatures remained below 11 C. Indices of maturity were highest for males and females on May 12 and 19, respectively, and by June 9 were lower than those recorded on April 28 (Fig. 2B). It is concluded that most of the spawning in 1971 occurred during the last half of May.

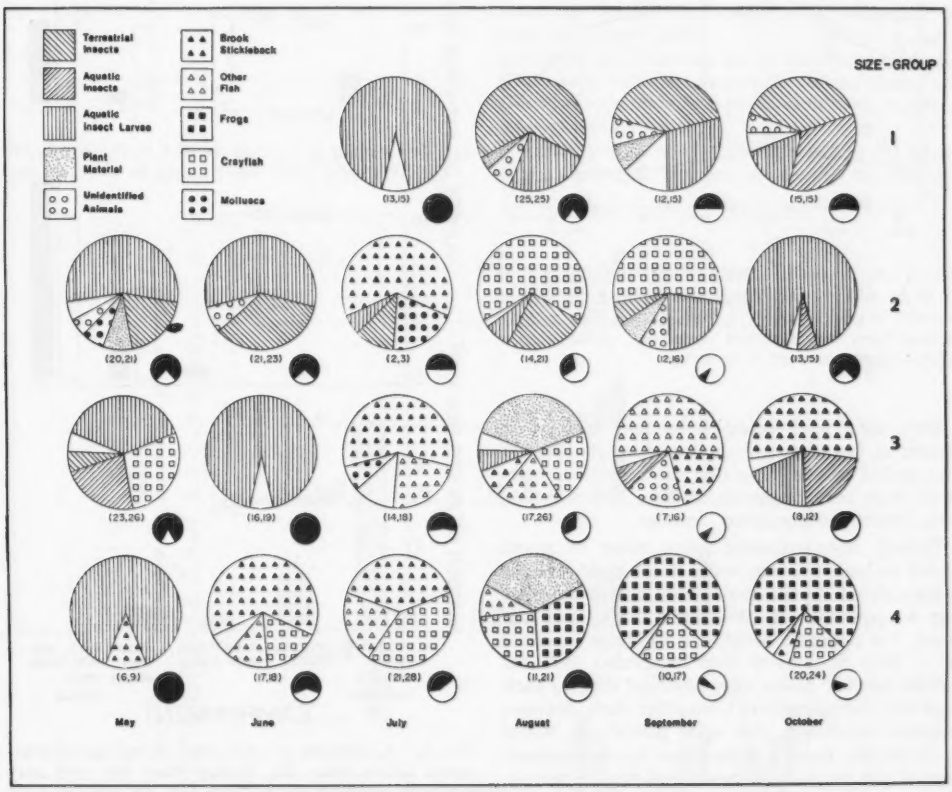

FIG. 1. Diet of creek chub by size-group, May–October 1970. Large circles, percentage weights of food items; open sections of large circles, items contributing less than 5% of total weight of food; smaller circles, percentages of fish (solid portion) containing the major item of food; numerals in parentheses, numbers of stomachs with food and total numbers examined.

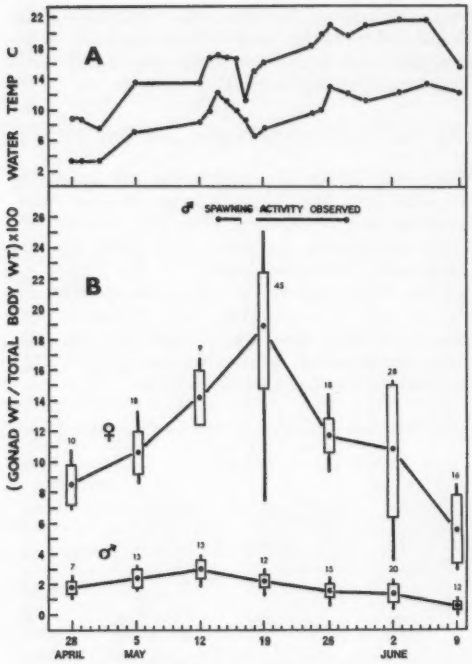

FIG. 2. A, ranges of surface water temperature over 24 hr in middle zone, April–June 1971. B, indices of maturity of adult chub, 1971. Vertical lines show ranges; vertical bars, two standard deviations; solid circles, means; numerals, sizes of samples.

Nest sites were conspicuous and most were located in fast and slow shallow channels (Fig. 3). The period of spawning was the only time when adult chub were numerous in these environments; here, males outnumbered females.

Typical nests consisted of a ridge of gravel (0.5–2 m long, 0.25 m wide, 5 cm high) parallel to the current. At the downstream end of the ridge was a spawning pit (20–25 cm diam, 8–20 cm deep). The substrate composing the ridge consisted of $>$ 50% fine gravel (See Moshenko 1972 for further details). Males were observed digging their nests and defending them from other chub. Between repeated spawnings, the male picked up stones in its mouth, moving them from the downstream edge of the pit to cover newly-laid eggs in the pit. This extended the gravel ridge downstream.

Number of mature ova per female was correlated with length ($r = 0.901$) and varied from 1146 to 7539 (Fig. 4).

ENVIRONMENTS OCCUPIED

The average density of chub in the middle zone (20.6/10 m^2) exceeded that of the upper zone (11.6/10 m^2), and they were rare in the lower zone (0.2/10 m^2). Within the middle zone, chub of the different size-groups occupied different environments at different times of the year. Size-group

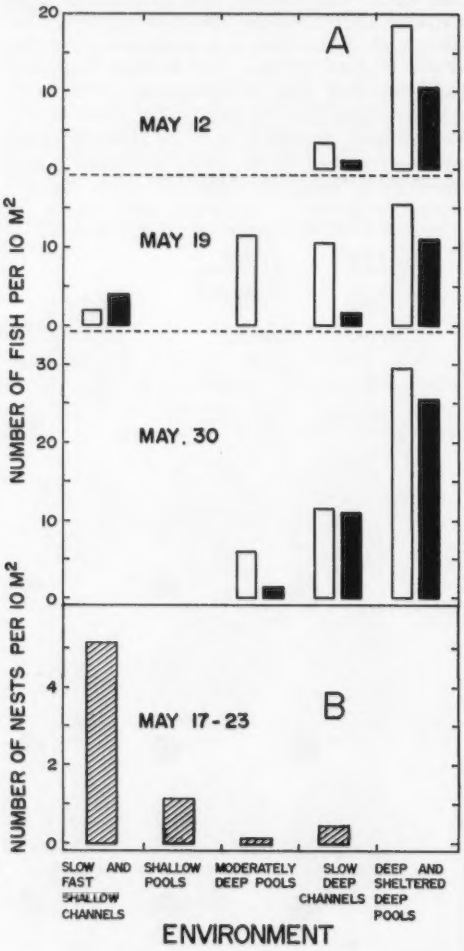

FIG. 3. A, densities of adult chub in various environments before (May 12), during (May 19), and after (May 30) spawning, 1971. Open bars are females, solid bars are males. Area sampled in each environment ranged 16–84 m^2. B, density of nests in various environments. From 33 to 1050 m^2 was searched in each combination of environment.

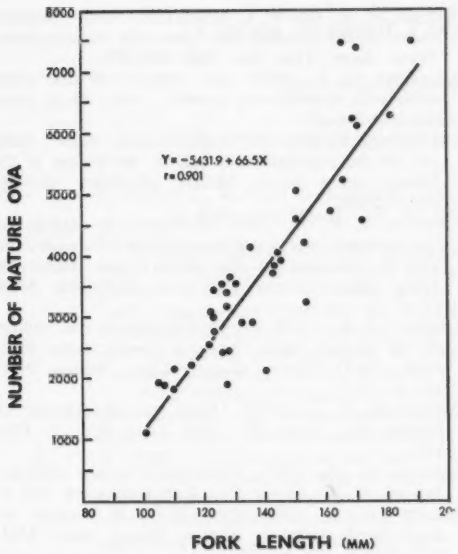

FIG. 4. Relation between number of mature ova and fork length in 40 gravid creek chub, May 1971.

1 was first observed in early July in shallow pools. Later in summer they were found mostly in slow shallow channels but, in October, they were most abundant in margins of deep and sheltered deep pools (Fig. 5). Size-group 2 was most dense in moderately deep pools from May to September and in slow deep channels in October. During May and June size-group 3 was most abundant in slow deep channels and deep and sheltered deep pools but some, particularly males, were found in fast shallow channels. Later in summer they were most dense in slow deep channels while, in October, most were found in deep pools. Chub in size-group 4 were most abundant in slow deep channels and deep pools from May to September except in June, when many were found in fast shallow channels. In 1970, when these data were collected, cool weather apparently delayed spawning into June. In October they were most abundant in sheltered deep pools.

Gillnets set under the ice in December captured 62 chub in sheltered deep pools and no chub were taken in moderately deep pools, slow deep channels, or deep pools.

Movements of marked fish, studied in the upper zone, confirmed changes in density in different

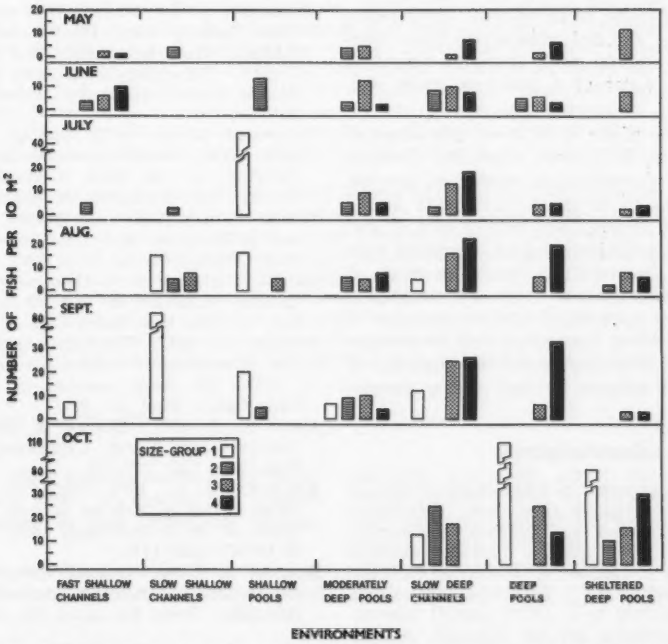

FIG. 5. Densities of creek chub in various environments of the middle zone, May–October 1970.

environments between seasons. In August, 345 chub (size-groups 2–4) were captured from slow deep channels and deep pools, marked, and released. From September to November, 18 were recaptured in slow deep channels and deep pools, while 39 were taken in sheltered deep pools. The mean distance moved was 0.78 km. Early in May, prior to spawning, 222 chub (size-groups 3–4) were captured from sheltered deep pools and marked. During spawning in May and early June, 17 were recaptured in slow shallow channels and 15 in slow deep channels and deep and sheltered deep pools. The average distance moved was 0.24 km. During July and August an additional 30 were recaptured, all in deep and sheltered deep pools. The mean distance moved was 0.42 km (See Moshenko 1972 for further details). Fast shallow channels, the environment where most spawning occurred in the middle zone, were lacking in the upper zone. There was no detectable trend in direction either up or downstream associated with these movements between seasons. Hankinson (1920) suggested that chub moved considerable distances prior to and after spawning. Possibly the presence of beaver dams in the upper zone restricted movements of fish.

In winter chub are faced with low water temperatures restricting speed of movement, the presence of mink, and a reduced volume of flow. Deep sheltered pools, where most chub are found in winter, provide water of a sufficient depth with hiding places.

The lower zone of the Mink River, characterized by a low gradient, few aquatic organisms (Gibbons 1971), and lack of overhanging vegetation, provides little in the way of either food or cover during winter for chub. Hence, their density here is low. In the upper zone the low gradient, combined with the presence of beaver dams, results in much of the bottom being covered with silt, restricting areas suitable for spawning. However, in terms of resources for feeding, spawning, and overwintering, the middle zone best meets the demands of chub, and this is reflected by their greater density.

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Copper Induced Lesions in Estuarine Teleosts

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Cellular changes attributable to copper were observed in the mechanoreceptors of the lateral line canals in the head of adult mummichog (*Fundulus heteroclitus*) and Atlantic silverside (*Menidia menidia*). The epithelium of these canals was also altered in *F. heteroclitus*. In both species, lesions were observed in the olfactory organs, which included the chemoreceptive sites. These manifestations of copper poisoning were evident at all studied concentrations (0.5, 1.0, and 5.0 mg/liter) of the metal. In *M. menidia* dilation of blood vessels was apparent, and in five cases hemorrhage had occurred in the brain and in periorbital connective tissues.

Renal lesions in *F. heteroclitus* exposed to 1.0 and 5.0 mg/liter of copper were apparent; these changes could not be identified in *M. menidia*. Hepatic changes were not detectable by light microscopy in either species following external exposures to copper. High concentrations of copper administered intraperitoneally to *F. heteroclitus* did induce liver damage.

Fry of *F. heteroclitus* were more sensitive to copper than were the adults or their zygotes. The emergence of larval forms from the zygote, the time required for emergence, and their survival was impaired by the metal. Lesions were not evident in developing sensory areas of the lateral line or the olfactory systems in these immature forms.

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Le cuivre dans un milieu salin produit des changements cellulaires de structures sensorielles au sein des extensions de la ligne latérale de la région céphalique de *Fundulus heteroclitus* et *Menidia menidia*. L'épithélium qui couvre l'intérieur de ces canaux est également affecté chez *F. heteroclitus*. Ces deux espèces démontrent des lésions au niveau des papilles chémoréceptrices qui forment l'organe olfactif des poissons. Ces changements associés à la présence de cuivre dans le milieu se manifestent à toutes les concentrations étudiées (0.5, 1.0 et 5.0 mg/litre). En plus, nous avons noté chez *M. menidia* des dilatations vasculaires, et dans cinq cas des hémorragies cérébrales et périorbitaires accompagnent les changements décrits plus haut.

Des lésions rénales sont apparentes chez *F. heteroclitus* exposés à des concentrations de 1.0 et 5.0 mg/litre de cuivre; chez *M. menidia* ces lésions ne peuvent être décelées. La présence de cuivre dans le milieu salin ne produit aucune anomalie hépatique décelable par l'examen histologique usuel. Chez *F. heteroclitus* les plus hautes concentrations de cuivre administrées par voie intrapéritonéalle causent des changements histologiques au niveau du foie.

Les jeunes *F. heteroclitus* semblent plus affectés par le cuivre que les adultes ou même les oeufs fertilisés. L'éclosion des oeufs et la survie des jeunes semblent affectées par la présence de cuivre. L'examen microscopique d'organes sensoriels et olfactifs de jeunes *F. heteroclitus* exposés aux concentrations de cuivre indiquées plus haut ne révèle aucune anomalie histologique.

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IN teleosts and other aquatic fauna, as in higher vertebrates, toxic levels of copper may initiate functional derangement and anatomical change, although knowledge about these events in the teleost remain sparse. Hepatic and renal disorders have been reported for the winter flounder, *Pseudopleuronectes americanus* (Baker 1969), while the metal was described as being both nephrotoxic and neurotoxic to the goldfish, *Carassius auratus* (Vogel 1959). The element also induced hepatic changes in the mummichog, *Fundulus heteroclitus* (Calventi and Nigrelli 1961).

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The behavioral reactions of these teleosts as influenced by copper suggests that the metal does exert a neurotoxic effect and consequent irritation to the perceptive system. Preliminary studies with *F. heteroclitus* have indicated distinct lateral line damage with copper exposure (Eisler and Gardner 1972; LaRoche et al. 1971). The purpose of the present study was to assess the extent of damage to the cephalic lateral line and the olfactory systems of *F. heteroclitus* and Atlantic silverside (*Menidia menidia*), two estuarine teleost species. In addition, the study was designed to evaluate the potential effects of the metal to early life stages of *F. heteroclitus*.

Materials and Methods

A total of 312 adult mummichogs, 140 advanced fry (yolk sac absorbed), and 140 zygotes, along with 36 adult Atlantic silversides, were utilized during the present study. Adults were collected in local Rhode Island estuarine waters.

The advanced fry and zygotes of *Fundulus*, and adult *Menidia* were exposed to copper in their aquatic medium. Adult *Fundulus* were exposed to copper in their aquatic medium and by means of intraperitoneal injection. Reagent grade copper chloride was used in all instances.

The initial copper concentrations to which adult (both species) and fry were exposed in their aquatic media were 0.0, 0.5, 1.0, and 5.0 mg/liter. In the portions of the study employing external exposures, the metal was added to the media following the introduction of fish. Control organisms were maintained under the prevailing environmental conditions of the experiment. The exposure of zygotes was at concentrations of 0.0, 0.25, 0.5, 1.0, 3.0, 5.0, and 10.0 mg/liter. The intraperitoneal injections were given to adults at concentrations of 0.0, 0.5, 1.0, 10.0, 20.0, and 100.0 mg/liter in a 0.05 ml volume of 0.7% saline. Control fishes received 0.05 ml of saline.

Adults were randomly selected from a population that had been maintained in laboratory holding facilities for 7 days or more. Zygotes, obtained through artificial spawning methods, were reared to advanced fry in 1000-ml glass culture dishes. All holding, rearing, and exposures were conducted in 20 C sea water (natural) adjusted to 20 ‰ salinity with fresh water.

The test aquaria for adult and larval fishes consisted of glass jars containing 2 liters of the above salt water. Plastic petri dishes (60 × 15 mm) served as test containers for the exposure of zygotes. Adult specimens (both species) were limited to three per aquarium, while 40 advanced fry were tested at each concentration. Three adult *Fundulus* were removed from each concentration and sacrificed at intervals of 1, 2, 3, 5, 7, 14, and 21 days. The media and the copper concentrations were renewed at 7 and 14 days. Five fry were to be removed and sacrificed at intervals of 1, 2, 3, and 7 days. However, high mortality at 1.0 and 5.0 mg/liter did not permit histopathological examination at all four intervals. *Menidia* were removed when moribund, or after 96 hr.

Eighty-four adult *Fundulus* were administered 0.0, 0.5, 1.0, and 5.0 mg/liter of copper via intraperitoneal injection at intervals of 1, 2, 3, 5, 7, 14, and 21 days. Twelve adults represented each interval, and 3 each concentration. Each individual received a single injection at the first interval (day 1). A maximum of seven injections were given to adults representing the seventh interval (day 21). These adults were sacrificed 24 hr after their final injection.

One-hundred-twenty adult *Fundulus* were intraperitoneally injected with 0.0, 10.0, 20.0, and 100.0 mg/liter of copper at intervals of 1, 2, 3, 5, and 7 days. One group of 60 adults received a single injection of each concentration and the second received two. Again, 12 adult *Fundulus* represented each interval and three each concentration. These adults were sacrificed 24 hr after exposure with respect to each interval.

The zygotes (20 per concentration) were exposed to copper within 24 hr after fertilization. The aquatic media (20 C and 20 ‰ salinity) was first changed at 15 days after fertilization and every 3 days thereafter. Following 24 days after fertilization the surviving sac fry and zygotes were fixed for histological examination.

All fishes were immediately immersed in Dietrich's fixative upon removal from an experiment and prepared for routine histological processing (Gardner and Yevich 1970). Adult specimens were transected near the pectoral fins, after which two sagital sections of the head were made in a longitudinal plane on a level approximate to the base of the orbits. The trunk was sectioned on the same plane medially. Twenty-four adult mummichogs were fixed in buffered formalin to demonstrate nerve fibers stained by a modification of Bodian's Technique (Chen and Chen 1969). Twelve of these adults were exposed 24 hr to 5.0 mg/liter of copper in the aquatic media, and 12 served as controls.

Larvae and zygotes were fixed in Dietrich's fixative for 30 min and placed in a dehydrant prior to processing. Tissue sections of adults were cut at 6 μ; larvae and zygotes were serial sectioned. Tissue sections were stained with Harris' Hematoxylin and Eosin.

Results

Lesions were observed in the mechanoreceptors of the cephalic extension of the lateral line, and the olfactory organs of adult *Fundulus* and *Menidia* (Fig. 1 and 2). These lesions were present in all experimental groups in which adults were exposed to copper in their aquatic media, regardless of time or concentration. The incidence and degree of alteration to sensory organs was related to copper concentration (Table 1).

The receptor or sensory cells of mechano receptors sustained substantial damage in both species. These cells displayed various degrees of degeneration as indicated by nuclear pyknosis and karyorrhexis, and a cytoplasmic alteration

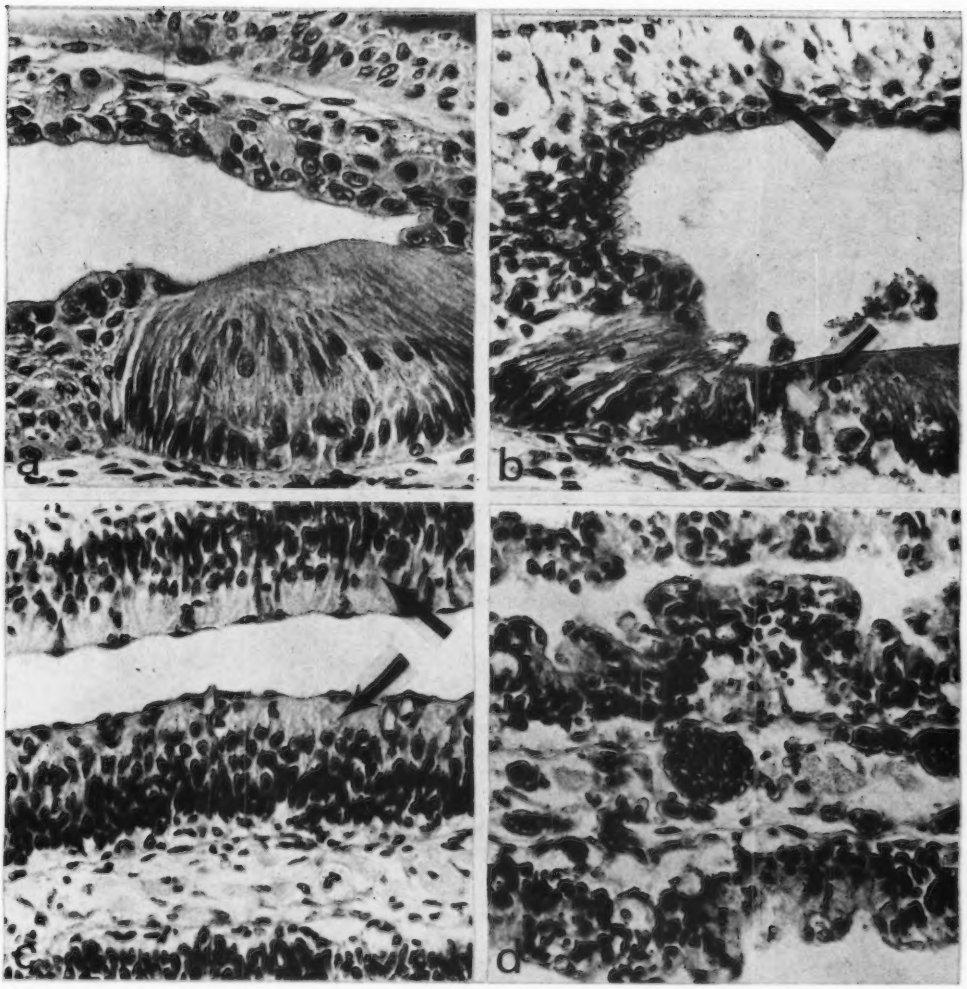

FIG. 1. (a) Control mechanoreceptor and mandibular canal of mummichog (*Fundulus heteroclitus*) (H&E × 160); (b) a location representative of Fig. 1a after 0.5 mg/liter of copper, 1 day (H&E × 160); (c) control olfactory lamellae and chemoreceptors (arrows) of Atlantic silverside (*Menidia menidia*) (H&E × 160); (d) olfactory lamellae of *Menidia* 0.5 mg/liter of copper, 6 hr; note congestion of vessels (H&E × 160).

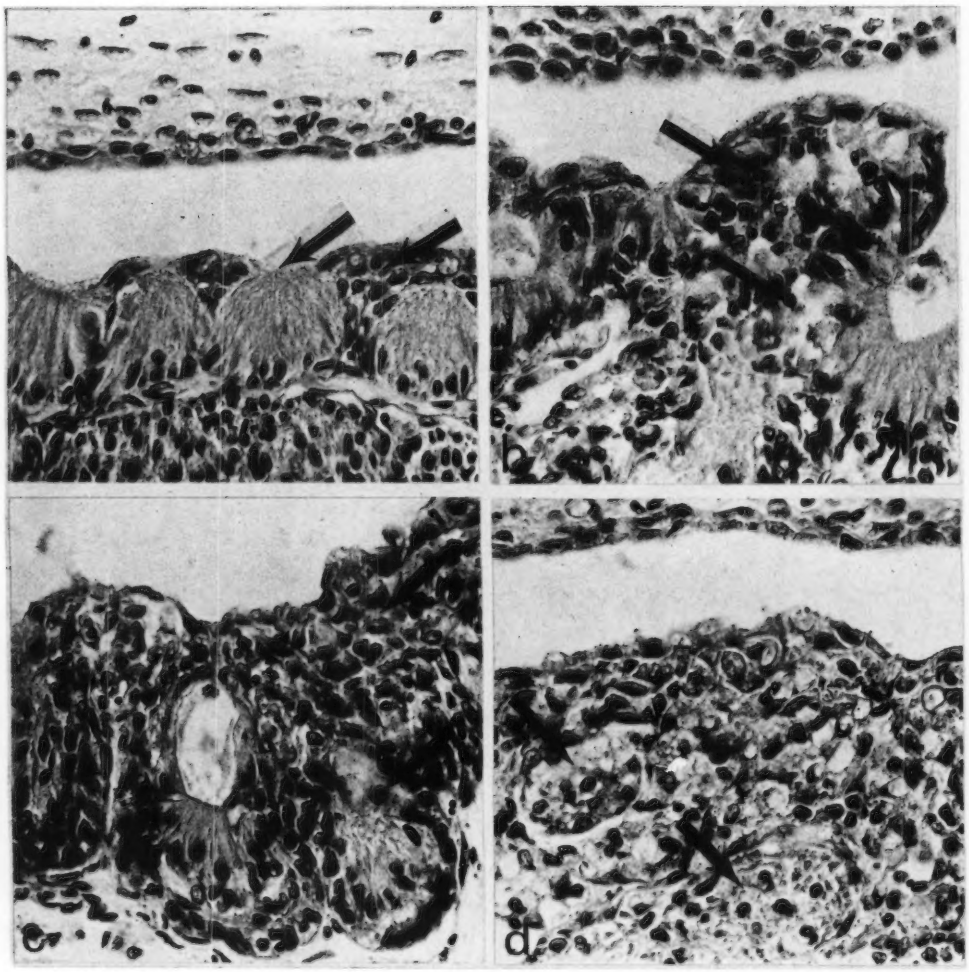

FIG. 2. (a) Control olfactory organ of *Fundulus*, note unaltered chemoreceptor sites and intersensory epithelium (arrows) (H&E × 160); (b) 0.5 mg/liter of copper, 24 hr. Note necrotized chemoreceptors and hyperplastic sustentacular epithelium (arrows) (H&E × 160); (c) as in Fig. 2b after 21 days. Note cystic formations (arrows) (H&E × 160); (d) 1 mg/liter of copper, 5 days (H&E × 160).

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TABLE 1. Incidence of lesions in groups of adult mummichogs (*Fundulus heteroclitus*) exposed to copper at concentrations of 0.5, 1.0, and 5.0 mg/liter for intervals up to 21 days. Column *a* represents number of animals per group (maximum 3) and column *b* is number of animals with the lesion.

	Epithelium						Mechanoreceptor						Chemoreceptor					
	0.5		1.0		5.0		0.5		1.0		5.0		0.5		1.0		5.0	
Days	*a*	*b*	*a*	*b*	*a*	*b*	*a*	*b*	*a*	*b*	*a*	*b*	*a*	*b*	*a*	*b*	*a*	*b*
1	3	2	3	2	3	3	2	1	1	1	2	2	1	1	2	2	3	3
2	3	1	3	1	3	3	3	2	3	2	2	2	2	2	1	1	2	2
3	3	3	3	3	3	3	3	0	2	2	3	3	–	–	2	2	2	2
5	2	1	2	1	3	3	3	3	1	1	2	2	2	0	3	3	2	2
7	3	3	3	3	3	3	1	1	2	2	2	2	2	1	2	2	2	2
14	3	2	2	1	–	–	3	3	2	2	–	–	3	3	1	1	1	1
21	3	2	3	1	–	–	3	2	–	–	–	–	3	3	–	–	1	1

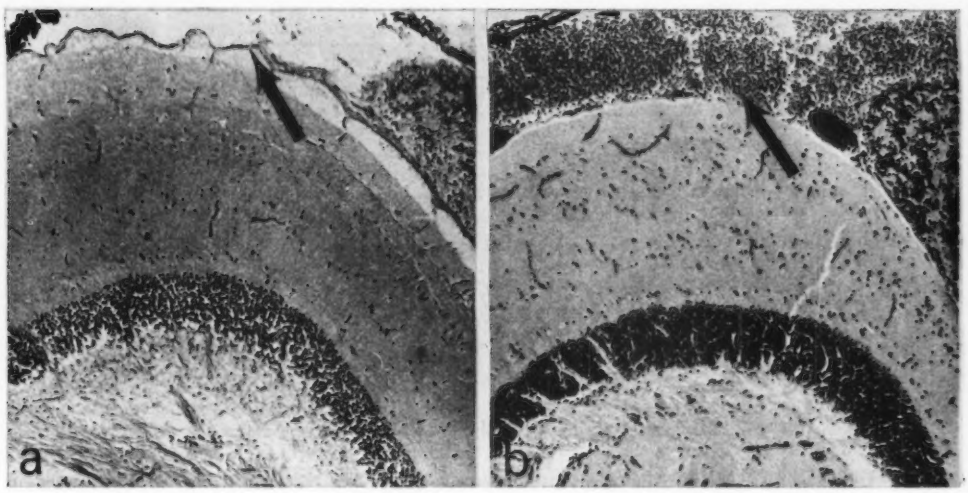

FIG. 3. (a) Cerebral hemisphere control of *Menidia*, note meninx (arrow); (b) vasodilation in meninx led to ruptures and hemorrhage (arrow), 1 mg/liter of copper (H&E × 160).

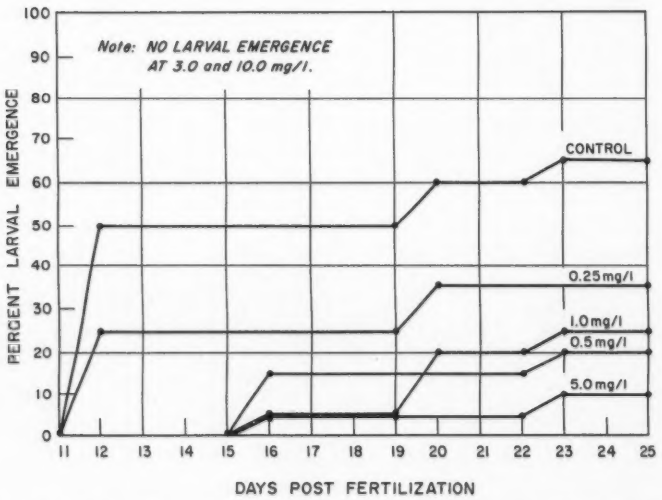

FIG. 4. Effects of copper on the emergence and survival of *Fundulus* larvae.

first occurring as a basally located swelling of the cells (Fig. 1a–b). The swelling extended to apical portions of the cells with exposure to 1.0 and 5.0 mg/liter of copper. The staining capacity of the cytoplasm in these cells was reduced prior to the disruption of cellular membranes. The supporting cells of these organs were also necrotized. In spite of these changes, the vasculature adjacent to organs was not visibly affected. The extent of damage to nerve fibers leading to organs could not be determined.

In *Fundulus*, the epithelium underlying the canal lining was swollen and necrotized. The change imparted an appearance of increased cellularity and, in most cases, reduced the luminal dimensions. The cuboidal cells lining the canal were not visibly altered by the metal. The usual complement of goblet cells in the canal epithelium was either depleted or lacking; however, secretory cells apparently were not affected.

The epidermal or free sensory organs located on the head and trunk were not affected by copper exposures, except for two cases in *Menidia* and one in *Fundulus*. The examination of epidermal organs, however, was limited due to an attempt to obtain as many canal organs as possible for examination. The lesions associated with the canal epithelium and organs of *Fundulus* exposed to copper externally could not be induced by interperitoneal injection of the metal. Damage to the lateral line of *Fundulus* fry could not be demonstrated by the present study.

The chemoreceptor sites of olfactory organs in adult *Fundulus* and *Menidia* were damaged after exposure to all external concentrations of copper. The architecture of the olfactory organs in *Menidia* (receptor cells, sustentacular cells, basal cells, and goblet cells) was essentially destroyed after 6 hr by the toxic action of 0.5 mg/liter or more of copper (Fig. 1d). Necrocytosis of the mucosa was accompanied by vasodilation and congestion of vessels in the submucosa. Vasodilation and congestion also occurred in all of the principal structures of the brain. Hemorrhage had occurred in the periorbital connective tissues and the principal structures of the brain with rupture of the meninx in five of these cases (Fig. 3a–b).

Lesions occurred in the olfactory organs of *Fundulus* after 24 hr exposure to 0.5 mg/liter of copper (Fig. 2b). The severity or progression of the lesion was in conjunction with length of exposure (Fig. 2c) and was noticeably more advanced with exposure to 1.0 and 5.0 mg/liter of the metal at corresponding intervals (Fig. 2d). The receptor cells of the olfactory epithelium in *Fundulus*, like those of *Menidia*, were necrotized; however, hyperplasia of the intersensory or sustentacular cells in *Fundulus* reduced or eliminated the olfactory surface by overgrowth. The enclosure and simultaneous deterioration of receptor cells formed cyst-like structures that contained cellular debris and remnants of recognizable sensory tissue. Injury to the olfactory placode of fry *Fundulus* could not be ascertained by light microscopy.

There was no visible evidence of hepatic alteration in either species exposed to copper in the aquatic media, although lesions were present in some *Fundulus* receiving paired injections of 100 mg/liter copper. The liver was marked by a few focal areas of necrosis in these cases. No renal alterations were associated with copper exposures to *Menidia*; however, *Fundulus* incurred renal damage after external exposures to 1.0 and 5.0 mg/liter of the metal (refer to Eisler and Gardner 1972). No renal lesions were associated with intraperitoneal injections.

The emergence of sac fry from zygotes occurred 12 days post fertilization in experimental groups receiving 0.0 and 0.25 mg/liter of copper (Fig. 4). Hatching occurred 4 days later in experimental groups receiving 0.5, 1.0, and 5.0 mg/liter. No hatching occurred in those groups receiving 3.0 and 10.0 mg/liter. A lag time for emergence was substantially increased with increased levels of copper and length of exposure. Upon termination of the experiment, the survival of hatched larvae was as follows: concentration 0.0 mg/liter, 100%; 0.25 mg/liter, 57%; 0.5 mg/liter, 75%; and 0% at all higher concentrations. At a concentration of 1.0 mg/liter or higher death followed emergence almost immediately; these fish were apparently incapable of gaining complete freedom from the egg casing.

The lethal concentration required to kill 50% of the larvae exposed to 0.5, 1.0, and 5.0 mg/liter of copper was established at 2.1 mg/liter (24 hr), 1.2 mg/liter (48 hr), and 1.0 mg/liter (72 hr).

Discussion

The unique and insidious damage to mechano- and chemoreceptive structures in *Fundulus* and *Menidia* suggests a vulnerability to copper that could conceivably have far reaching repercussions on the survival of a species. Although a brief encounter with a pollutant such as copper may not cause immediate death, it is apparent it could indirectly promote lethality by disrupting a species ability to perceive peripheral events.

The ability to perceive peripheral events such as the location of social partners, enemy, and prey is reliant, among other sensations, upon the lateral

line attuned to the detection of underwater vibrations and motions (Dijkgraaf 1963), and chemoreception encompassing olfaction, gustation, and other chemical sensations (Hara 1971; Atema et al. 1969). The behavior of fishes during migration, reproduction, feeding, schooling, and other responses compatible with survival are also dependent upon these systems (Atema et al. 1971; Flock 1971; Hara 1971; Tavolga 1971; Dijkgraaf 1963; Harris and Bergeijk 1962).

The functions of various sensory arrangements in the teleost are intimately interrelated. A unique interrelation between the eyes and the cephalic lateral line canal organs in the mummichog is evident during feeding as interpreted by Harris and Bergeijk (1962). These authors have described the presence of a "blind cone" extending away from the front of the head and mouth of these fishes owing to the anatomical arrangement of their eyes. The initial sensation while feeding on prey is established by sight; however, once the prey enters into the area of the "blind cone", visual contact is lost. The correlation of the activity is then relegated to the canal organs that are used to zero in on the prey. Olfaction may be involved in the culmination of the process after location by the above means (Hara 1971). The combined events of injury to the lateral line and olfactory organs that were induced in the mummichog by copper exemplify one manner by which a normal behavior pattern could be seriously disrupted and survival threatened. The impairment would not necessarily be limited to the above behavior pattern, but may also extend to the detection of body odors necessary to schooling, orientation, migration, alarm substances, and repellants.

Available evidence indicates that minute amounts of some toxicants have the ability to cause abnormal behavior performance, and, although impaired perceptive acuity may have been responsible, the possibility of injury to sensory tissue was not determined. Carp in fresh water, for instance, reduced their food consumption by 30% during a 30-day exposure to 8 µg/liter of copper (Ozaki et al. 1970), and the metal inhibited the spawning of adult fathead minnows (*Pimephales promelas*) during long term chronic exposures in fresh water (Mount 1968; Mount and Stephan 1969). The apparent fast and the inhibited spawning could conceivably have been accompanied by histological anomalies similar to those of the present study. The heavy metals arsenic, lead, mercury, and selenium reduced the behavioral performance of conditioned goldfish (*C. auratus*) (Weir and Hine 1970), which obviously may relate to their inability to correlate sensations.

Toxic compounds other than heavy metals can visibly influence behavioral responses. Anderson (1968) indicated that brook trout (*Salvelinus fontinalis*) swimming in horizontal temperature gradients during exposure to sublethal concentrations of DDT exhibit violent avoidance reactions when encountering low water temperature. The response was related to the lateral line, although evidence of injury to the system was not reported. It is significant to note that evidence of stress can be detected by monitoring neural discharges of the lateral line in rainbow trout (*Salmo gairdneri*) exposed to cyanide (Bahr 1971).

The preliminary studies of Atema et al. (1971) indicate that certain petroleum products affect chemotaction and, ultimately, the behavior of several marine organisms. Therefore, a variety of water pollutants can induce symptoms of behavioral stress that may relate to chemical or morphological anomalies that have reduced their ability to correlate sensations.

The recent investigations of Gardner and LaRoche (1972) strengthen the probability that damage to perceptive structures occurred in the above instances. The heavy metals mercury and silver and the pesticide methoxychlor induced lesions in the lateral line and olfactory organs of *Fundulus*, while a whole crude oil and the soluble and insoluble fractions of the whole crude oil induced lesions in the olfactory organs of *Menidia* during laboratory experimentation. Notably, the heavy metals cadmium and zinc did not cause these lesions in *Fundulus*. Atlantic menhaden (*Brevoortia tyrannus*) stressed in their natural marine environment had lesions associated with the lateral line, the olfactory organs, and the internal ear or membranous labyrinth.

Intraperitoneal injection of copper into mummichogs, unlike external exposure to the metal, did not cause lesions in the lateral line or olfactory organs. These lesions, therefore, are seemingly the consequence of a direct irritation by the metal and not indirectly via a rise in the vascular system. However, intoxication assumed via an internal exposure route cannot be precluded until it can be endorsed by further research.

The present study could not confirm visible hepatic lesions in either *Menidia* or *Fundulus* exposed to copper externally. However, liver disorders have been reported in the mummichog (Calventi and Nigrelli 1961) and the flounder *P. americanus* (Baker 1969) following copper exposures. The absence of hepatic lesions in mummichogs exposed to copper in their aquatic media during the present study may relate to specific exposure conditions or other environmental influences. However, it should

be mentioned that intraperitoneal injections of very high, unrealistic copper concentrations did cause limited hepatic necrosis in mummichogs during the present study. It might finally be indicated that liver cell functions can be affected long before the appearance of a cellular alteration that can be readily identified with a light microscope (Robbins 1967).

The results obtained after relatively short periods of exposure to copper suggest that the early life stages of *Fundulus*, from the zygote through advanced sac fry, are more sensitive to the acute toxic properties of the metal than are the adults. These preliminary studies also indicate that the sac fry were more sensitive to copper than were the fertilized eggs. These findings corroborate those of McKim and Benoit (1971), who reported impaired survival and growth of rainbow trout alevins–juveniles following long term copper exposures. The growth, survival, and spawning of adult rainbow trout and the ability of their eggs to hatch was not impaired by the metal. The results of the present study are also in accord with those of Hazel and Meith (1970), who found the eggs of king salmon to be more resistant to copper than were the fry.

The route of penetration taken by copper in adult *Fundulus* and *Menidia*, and the nature of the lesions associated with the penetration have extraordinary import. Impaired behavioral performance, as previously mentioned, can be influenced by a variety of toxicants, indicating that biochemical or morphological lesions in the sensory mechanisms of teleosts may be widespread. The character of the spontaneous lesions in equilibratory and perceptive organs of the menhaden adds impact to the prospect. Therefore, it is logical that these effects may be far-reaching if a significant number of individuals become incapable of coping with a polluted environment.

Acknowledgments

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Sublethal Effects of Bleached Kraft Pulp Mill Effluent on Respiration and Circulation in Sockeye Salmon (*Oncorhynchus nerka*)[1]

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DAVIS, JOHN C. 1973. Sublethal effects of bleached kraft pulp mill effluent on respiration and circulation in sockeye salmon, (*Oncorhynchus nerka*). J. Fish. Res. Board Can. 30: 369–377.

Sublethal effects of aerated neutralized, filtered, full bleach kraft mill effluent (BKME) on circulation and respiration of Pacific salmon were examined. Ventilatory water flow, oxygen uptake, cough frequency, and buccal pressure increased in a group of 19 sockeye salmon, *Oncorhynchus nerka*, 207–321 g, at 10.5 ± 0.5 C, upon initial exposure to sublethal BKME concentrations. The threshold concentration for these responses appeared to be around 20% of the 4 day LC50 (static bioassay).

Following overnight exposure to BKME, ventilatory volume, oxygen uptake rate, cough frequency, and oxygen utilization tended to approach pre-exposure levels, particularly at the higher sublethal contractions. Changing effluent toxicity, acclimation phenomena, or physiological adjustment are discussed as possible explanations for these results.

Measures of arterial oxygen tension in sockeye salmon indicated that arterial tension declines rapidly and remains depressed following up to 24 hr exposure to BKME (33–47% of 4 day LC50). On the average this decline represented a 20% decrease in oxygen saturation of the blood. Decreased arterial PO_2 may be due to mucous production at the gills and resulting gas diffusion problems, as well as abnormalities in ventilation. Reduction in scope for activity might result from impaired oxygen uptake at the gills. A similar response was observed in rainbow trout, *Salmo gairdneri*.

DAVIS, JOHN C. 1973. Sublethal effects of bleached kraft pulp mill effluent on respiration and circulation in sockeye salmon, (*Oncorhynchus nerka*). J. Fish. Res. Board Can. 30: 369–377.

L'auteur étudie les effets sublétaux de l'effluent de moulin de pâte à papier kraft (BKME), complètement blanchi, filtré, neutralisé et aéré, sur la respiration et la circulation des saumons du Pacifique. L'écoulement d'eau de ventilation, la fixation de l'oxygène, la fréquence de la toux, et la pression buccale augmentent chez un groupe de 19 saumons nerka, *Oncorhynchus nerka*, pesant 207–321 g, à 10.5 ± 0.5 C, après exposition initiale à des concentrations sublétales de BKME. Le seuil de concentration de ces réponses semble être aux environs de 20% de la LC50 (4 jours) (essai biologique statique).

Après exposition d'une nuit au BKME, le volume ventilatoire, le taux de fixation de l'oxygène, la fréquence de la toux, et l'utilisation de l'oxygène ont tendance à se rapprocher des niveaux d'avant l'exposition, surtout aux plus fortes concentrations sublétales. Comme explications possibles de ces résultats, l'auteur considère une toxicité variable de l'effluent, des phénomènes d'acclimatation, ou un ajustement physiologique.

Les mesures de tension d'oxygène du sang artériel du saumon nerka indiquent que la tension diminue rapidement et demeure faible après une exposition allant jusqu'à 24 heures au BKME (33–47% de la LC50 après 4 jours). En moyenne, ce déclin correspond à une diminution de 20% du point de saturation de l'oxygène du sang. La diminution de la pression d'O_2 du sang artériel peut être due à la production de mucus au niveau des branchies, et aux problèmes de diffusion des gaz qui s'ensuivent, tout autant qu'à des anomalies de ventilation. Un obstacle à la fixation de l'oxygène sur les branchies peut causer une diminution du cadre d'activité de l'animal. Une réponse semblable a été observée chez la truite arc-en-ciel, *Salmo gairdneri*.

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ALTHOUGH a great deal is known of the acute toxicity of kraft pulp mill wastes to fish (Alderdice and Brett 1957; Betts and Wilson 1967; Haydu et al. 1952; Howard and Walden 1965; Servizi and Gordon 1966; Van Horn et al. 1949), very little is known of their sublethal effects. Waldichuk (1962) and Howard and Walden (1965) emphasized the need for better understanding of the sublethal effects of these wastes, to determine the concentration that would cause no deleterious effect on the species concerned. In a recent review, Sprague (1971) discussed sublethal effects of pollutants, emphasizing that in some instances, sublethal effects can be subtle, yet cause serious damage to fish populations.

In Canada, large quantities of kraft mill wastes are discharged into both tidal and freshwater frequented by salmon at various stages of their life-cycle. Much of this waste enters the environment in an untreated state. It is of importance then, that the sublethal effects of the waste be elucidated. This report describes experiments on circulation and respiration in Pacific salmon exposed to bleached kraft mill effluent.

Methods

EFFLUENT HANDLING PROCEDURES

Samples of full-bleach kraft pulp mill effluent (BKME) were collected from a local mill every 7–10 days. Effluent was collected by means of a pump from the alkaline waste sewer and bleach-plant wastes were obtained from sampling valves in the mill (chlorination effluent, 1st chlorine dioxide treatment, 2nd chlorine dioxide treatment). Caustic extraction effluent was sampled along with the alkaline waste. The total sample volume collected over a ½-hr period varied from 150–400 imperial gallons. Samples were transported, unmixed, to the laboratory and stored in a cold room at 2 C for up to 10 days.

The mill sampled is a full-bleach kraft mill with a production capacity of 540 ADT (U.S.) per day of full bleach pulp. Water discharge from the process totals 50,000 gal/ton pulp produced, soda loss 100 lb/ton, fiber loss 15 lb/ton. The bleaching sequence is CEDED (chlorination, caustic extraction, chlorine dioxide, caustic extraction, chlorine dioxide treatment) with 130 lb/ton chlorine used during full bleach production. No treatment is practised and alkaline and bleach plant wastes are sewered separately. Wood pulped is usually a chip mixture of 25% western red cedar, 30% douglas fir, and 45% hemlock.

Samples were usually taken only of full bleach effluents associated with 88 brightness pulp. Occasionally semi-bleach effluents (70 brightness) were collected. Samples were only collected when the mill personnel felt the process was operating in a normal, trouble-free fashion.

Immediately before use, samples of effluent from the different waste streams were mixed together in the proportions approximating their discharge into the sea. Mill personnel reported that this mixture resembled as closely as possible, the composite effluent leaving the plant. The composite effluent was then aerated overnight to drive off volatile toxic components (Alderdice and Brett 1957; Gehm 1953; Howard and Walden 1965), and was filtered through pyrex glass wool and neutralized with 0.5 N NaOH or 0.5 N H_2SO_4 prior to use. The rationale behind these procedures was to eliminate volatile toxicants and *p*H effects and deal strictly with persistant toxic constituents that would tend to remain in receiving waters for long periods.

BIOASSAY PROCEDURES

Neutralized, filtered, pre-aerated composite effluent samples were tested for toxicity by means of 4-day static bioassay procedures (Sprague 1969). Continuously aerated, 45.5-liter, all glass aquaria were used, each with 10–12 underyearling coho salmon, *O. kisutch* for each test concentration. The relation between weight of fish tested and volume of test solution was 1–2 g fish/liter of test solution and all bioassays (and subsequent experiments) were conducted in freshwater of approximately 20 ppm hardness ($CaCo_3$). Over the experimental period water temperature varied from 8–12 C but never varied more than 0.5 C for any one bioassay or sublethal study carried out with an effluent sample. Dissolved oxygen in the test tanks was always between 80 and 100% of full saturation. Preliminary bioassays were usually carried out using six fish/concentration in a single tank to establish toxicity levels. Rigorous bioassays were subsequently done using two replicates of each test dilution, each with 10–12 fish.

Mortality in each test tank was recorded during the 4-day bioassay period. These data were converted to cumulative percentage mortality and plotted on probit paper for estimation of the time to 50% mortality at each different concentration. The time to death of 50% of the fish at each concentration was plotted on semi-log paper for estimation of the 4-day LC50 (Sprague 1969). For each effluent sample the 4-day LC50 was used as the standard solution from which sublethal BKME dilutions were prepared (Fig. 1).

To see whether the sensitivity of coho underyearlings and sockeye underyearlings to BKME differed comparative static bioassays were run. Fish density for both species was 3 g fish/liter of test solution at 10.5C. The 96 hr LC50 for sockeye was 32% full strength BKME and 35% for coho underyearlings. It would appear that there was no large difference in sensitivity of the two species to BKME. To my knowledge there is no evidence to suggest that adult sockeye are more or less sensitive to BKME than underyearlings. Hence, the following experiments were done on the assumption that BKME toxicity to adult fish was similar to underyearlings. This assumption requires further testing.

RESPIRATION STUDIES

Circulatory and respiratory responses of 19 3-year old sockeye salmon, *O. nerka*, 207–321 g, in freshwater at 10.5 ± 0.5 C, were studied before and after exposure

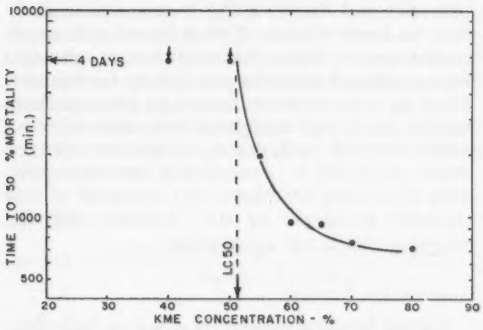

FIG. 1. A plot of the time to 50% mortality for test groups of coho underyearlings vs. effluent concentration. Arrows indicate that less than 50% mortality occurred in the test period.

to BKME. Fish were anesthetized with 1:12,000 M.S. 222 and placed on an operating table (Smith and Bell 1967), where a 30-cm Clay–Adams PE 160 buccal cannula was implanted similar to that described by Saunders (1961). In addition, a rubber membrane device was stitched to the mouth of each fish (Cameron and Davis 1970: Davis and Cameron 1970). This membrane separated inspired and expired water and allowed collection of the latter for direct measurement of ventilation volume (the amount of water in milliliters, pumped across the gills/minute, abbreviated $\dot{V}_G$). Following the operation, the fish were placed in the apparatus (Fig. 2) and allowed to recover overnight. This apparatus consisted of a 120-liter capacity temperature-controlled recirculation system capable of holding three fish at a time.

The experimental procedure consisted of recording buccal pressures and ventilation frequency (mouth closures/minute) with a pressure transducer system (Statham P23BB and Beckman RM Dynograph Recorder), inspired and expired water O_2 tensions (Radiometer blood gas analyser, PHM72), and ventilation volume, $\dot{V}_G$, before, during and after BKME exposure. Readings were taken for 3–4 hr while the fish remained in clean water. Water was then siphoned from the reservoir and replaced with an equivalent volume of neutralized, filtered BKME. Readings were repeated periodically for 3 hr after effluent addition, again after the fish had remained overnight in the effluent and for 3 hr following their return to freshwater. All readings were taken simultaneously with a time lag of a maximum of 3 min between measurement of any one ventilatory variable.

ARTERIAL OXYGEN TENSION MEASUREMENTS

A separate experiment was conducted on nine 3-year-old sockeye in freshwater to study the effect of kraft mill effluent on arterial blood oxygen tension. Fish ranging in size from 357–512 g were studied at temperatures of 12–14 C. Each salmon was anesthetized and equipped with a 60-cm PE60 dorsal aortic catheter as described by Smith and Bell (1964). The fish was then placed in an aerated, covered, darkened 45.5-liter aquarium for overnight recovery. The following day, blood samples were taken from each fish for oxygen tension measurement. Subsequently, some water from each tank was gently siphoned off (without disturbing the fish) and replaced with an equivalent volume of neutralized, filtered KME. Blood samples were taken periodically from each fish for up to 24 hr, while they remained in the sublethal effluent dose. Water oxygen tension was maintained between 140–150 mm PO_2 in every tank.

A blood curve relating oxygen saturation of the blood to oxygen tension was constructed using the mixing

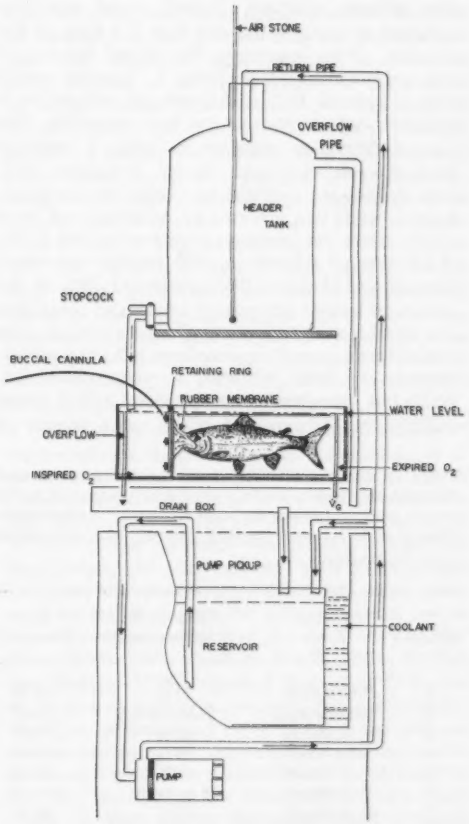

FIG. 2. The recirculation apparatus used to study ventilatory responses of sockeye salmon to kraft mill effluent. Temperature was controlled within ± 0.5 C by the cooling unit. Ventilation volume of the fish was measured by collecting the water spillage over the rear drain.

technique of Edwards and Martin (1966). Pooled blood samples from two adult sockeye salmon and a Radiometer oxygen electrode system were used to construct the curve. This blood curve was used for interpretation of the arterial oxygen tension experimental results.

Results and Discussion

Bioassays

Figure 1 represents typical bioassay results obtained with underyearling coho salmon. In every instance there was a fairly well-defined concentration at which acute mortality ceased even if the observation period was extended up to 7 days after effluent addition. Indeed, most mortality appeared to occur within the first 2–3 days of the bioassay. LC50s determined for several batches of KME are summarized in Table 1. Toxicity varied from a high of 37% of full-strength effluent to a complete lack of toxicity on two occasions. The 4-day LC50 values reported in Table 1 resemble those reported for three British Columbia kraft mills by Howard and Walden (1965) but are considerably below that reported by Alderdice and Brett (1957) where the limiting concentration was 4.8% of full-strength effluent in 20‰ salinity sea water. Howard and Walden (1965) attributed 75% of the toxicity of KME to adverse *p*H effects and concluded that Alderdice and Brett's high toxicity values were related to the use of unneutralized effluent samples. Seawater of 20‰, however, is well buffered (M. Waldichuk personal communication) and it seems unlikely that adverse *p*H effects were present in Alderdice and Brett's study. It seems more likely that the lower toxicity of BKME recorded in recent studies results from improved inplant chemical recovery procedures being practised by the industry. Since the 4-day LC50s reported here for neutralized samples are in good agreement with similar bioassay results obtained by Howard and Walden (1965), it seems reasonable to conclude that my experiments were done using an effluent that is typical of that presently discharged by B.C. Industry with the exception that it was air-stripped.

TABLE 1. Results of 4-day static bioassays conducted with effluent from a single coastal kraft pulp mill. LC50s are for groups of underyearling coho tested in freshwater. Effluent samples were aerated, filtered, and neutralized prior to bioassay.

Batch#	Effluent Type	4-day LC50 (% Full-strength)	Test Temp (C)
1	Full bleach	55%	12.0
2	Unbleached	Non-toxic	12.0
3	Full bleach	93%	11.0
4	Semi-bleach	55%	11.0
5	Full bleach	55%	11.5
6	Full bleach	Non-toxic	11.0
7	Full bleach	52%	10.5
8	Full bleach	37%	10.5
9	Full bleach	45%	11.0
10	Full bleach	55%	10.8
11	Full bleach	20%	11.0
12	Full bleach	Non-toxic	12.0
13	Full bleach	80%	12.0

Respiration Studies

Results of respiration studies before and after fish were exposed to various sublethal doses of BKME are summarized in Fig. 3. Within 2 hr of exposure, ventilation volume more than doubled over the range of sublethal concentrations tested and the threshold for this increase appeared to be about 20% of the LC50. After overnight exposure to BKME however, $\dot{V}_G$ dropped and was lowest in fish maintained in the strongest effluent solutions.

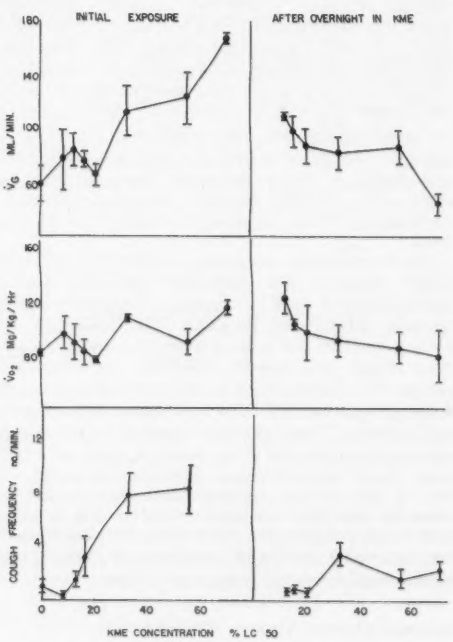

FIG. 3. Ventilatory water flows, cough responses, and oxygen uptake rates for a group of nineteen sockeye before and after BKME exposure. Initial exposure means recordings were made in the first 3 hr after effluent was added. Values are means ±2 SE.

Oxygen uptake rate ($\dot{V}O_2$) showed a similar trend, increasing by about 50% over the range of sublethal concentrations tested on initial exposure, and remaining elevated after overnight exposure only in fish exposed to low doses of BKME.

Utilization of oxygen from the water, the percentage of oxygen removed from the water by the fish, calculated from:

$$\% \text{ Utilization} = 100 \times \frac{(\text{Inspired } O_2 \text{ tension} - \text{Expired } O_2 \text{ tension})}{\text{Inspired } O_2 \text{ tension}} \quad (1)$$

was 42.5 ± 2.3% before exposure and dropped to a low of 32% at high sublethal doses of BKME on initial exposure. After overnight exposure to all sublethal BKME concentrations % U approximated the pre-exposure level.

Pressures in the buccal cavity recorded before exposure to effluent averaged 1.24/–0.8 mm Hg (maximum/minimum) and increased slightly in amplitude with sublethal dose on initial exposure. After overnight in BKME the amplitude of the pressure trace had increased considerably above pre-exposure levels, particularly in the fish in low to moderate sublethal doses.

Abnormal patterns of breathing with frequent "coughing" occurred on initial exposure to sublethal concentrations of 20% of the LC50 or higher (Fig. 4). After overnight in effluent however, cough frequency in all test doses was close to pre-exposure level. As cough frequency rose, normal ventilatory pressure pulses declined somewhat and had dropped from 57 ±2.2/min (pre-exposure) to 36/min at 70.5% of the LC50 after overnight exposure.

Behavior of the fish was fairly consistent. Typically, fish remained quiet during the pre-exposure period and showed little struggling or restless behavior in low sublethal doses. High sublethal doses, however, (> 20% LC50) produced struggling and restless behavior as evidenced by artifacts which appeared frequently on the buccal pressure trace due to cannula movement. This behavior alone could be a significant sublethal effect. Following return of the fish to freshwater most parameters measured tended to return to pre-exposure levels in all concentrations tested. After 3 hr in freshwater, ventilation volume, oxygen uptake rate and buccal pressures remained slightly above pre-exposure levels in fish that were exposed to 12.5, 16.4, and 33% of the LC50.

As a control several sockeye were run through the entire test procedure but no effluent was added to the system. In all cases respiratory and circulatory parameters remained at pre-exposure levels.

The threshold for respiratory responses appeared to be around 20% of the LC50. Davis and Cameron (1970) measured the ventilation volume ($\dot{V}_G$) of rainbow trout at 8.6 C and found that resting $\dot{V}_G$ averaged 37.0 ± 1.8 ml/min and that it could increase to nearly 300 ml/min during periods of activity. Since the sockeye salmon tested here were of similar size and tested at a similar temperature as Davis and Cameron's trout, then an increase in mean $\dot{V}_G$ from 62 ml/min to 169 ml/min observed over the range of effluent concentrations tested could represent a large increase in gill water movement, relative to the physiological range for $\dot{V}_G$. In addition, an increase in ventilatory work must have occurred, since more energy expenditure would be necessary to propel water over the gills (Alexander 1970). Elevated metabolic cost of breathing could account in part for a portion of the increase in oxygen uptake rate observed over the range of concentrations tested, however, branchial pumping appears a relatively efficient process (Cameron and Cech 1970; Davis and Randall 1973) and thus would not explain completely the large increase in oxygen uptake observed. The fish were definitely more restless in high sublethal BKME concentrations and this increase in activity and general muscle tone could have been sufficient to account for elevated oxygen uptake (Brett 1962). It is also possible that the effluent had a direct effect on metabolism, increasing the maintenance costs of body processes. Such a phenomenon has been termed a "loading stress" by Brett (1958).

Cough responses of the sockeye to BKME (Fig. 4) were similar to those reported by Schaumburg et al. (1967) and Walden et al. (1970). On initial exposure, coughing appeared to increase with BKME concentration above 20% of the LC50. Considerable variability in cough frequency was evident between fish, both before and after exposure to a given effluent concentration. Presumably, the cough reflex serves as a means of ridding the gills of foreign matter and eliminating clogging of the respiratory epithelium (Bijtel 1949; François–Frank 1906). Hughes and Shelton (1958) suggested that water flow over the gills is momentarily reversed during a "cough." The flow of water and blood through the gills are countercurrent to one another and a maximum oxygen tension gradient is maintained at the gill lamellae by this countercurrent arrangement (Van Dam 1938). A high cough frequency could seriously interfere with this flow arrangement due to water flow reversal and thus affect the efficiency of gas exchange. In addition, the gill filament tips, which interdigitate to form an effective sieve, are pulled wide apart by their intrinsic muscles during a

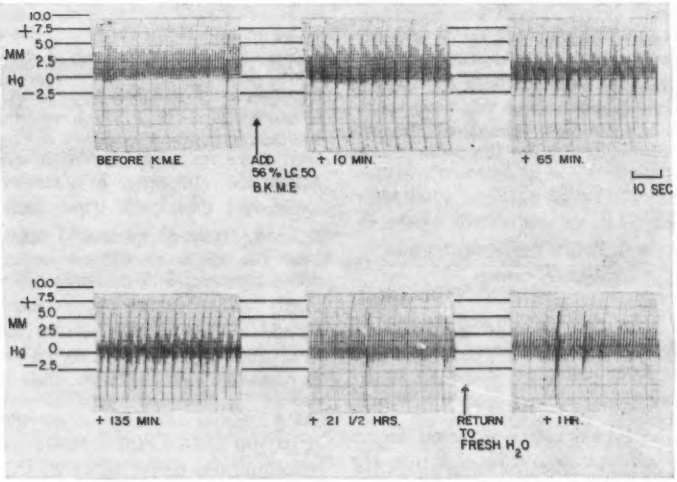

FIG. 4. Pressure recordings from a buccal catheter in a single fish (300 g) recorded before and after effluent addition. Large spikes are coughs. Some movement artifacts are evident as wide spikes (+ 65 min exposure, + 1 hr in freshwater).

cough (Bijtel 1949). High cough frequency, combined with elevated $\dot{V}_G$ and reduced residence time for water in the gills (Hughes 1966) could account for decreasing oxygen utilization with increasing effluent concentration observed on initial exposure to BKME.

In considering cough frequency, it is interesting to note that coughing appears to occur in response to chemical as well as mechanical stimuli. MacLeod and Smith (1966) described frequent coughing, impaired oxygen uptake, and reduced swimming ability in fathead minnows, *Pimephales promelas*, exposed to pulpwood fiber. This study, and that of Schaumburg et al. (1967) and Walden et al. (1970) used filtered effluent samples, hence the cough responses were probably related to chemical content in the water rather than particulate matter.

Following overnight in BKME ventilation volume, oxygen uptake rate, cough frequency, and oxygen utilization had dropped and tended to approach pre-exposure levels, particularly at the higher sublethal concentrations. These results could be explained in several ways:

1, that the toxicity of the effluent had changed;
2, that the fish had become acclimated to the effluent;
3, that the fish were responding to the maintained toxic stress by adjusting respiratory parameters.

There is insufficient data to conclusively account for such results. Pre-aeration of the effluent was carried out for 18–20 hr prior to testing so presumably the volatile, easily oxidized toxic components in the effluent were gone. Howard and Walden (1965) reported that salmon appear to become acclimated to kraft pulp mill bleach wastes and that pre-exposure to these wastes allowed survival in effluent concentrations lethal to unacclimated fish. They attributed acute mortality to the "shock" of initial exposure to high concentrations of effluent. Indeed, the responses observed, on initial exposure could be related to behavioral phenomena. Sprague and Drury (1969) demonstrated that salmonids can detect and avoid kraft pulp mill wastes. It is possible that the fish were able to detoxify the effluent to some degree. Bioassays (J. C. Davis and B. J. Mason unpublished data) have indicated that use of a large volume of fish relative to the volume fo test solution reduced toxicity. For reasons of economy of effluent the ratio of fish weight to effluent volume was around 6 g fish/liter of solution in the respiration studies. Lastly, the return of ventilatory responses towards pre-exposure levels may constitute responses of the fish in an attempt to conserve energy and maintain homeostasis in the presence of toxic stress. It is relevant that the return of $\dot{V}_G$ and $\dot{V}_{O_2}$ to pre-exposure levels was most evident in the highest sublethal concentrations tested — the concentrations where maximum regulation of response might be most useful in terms of conservation of energy.

The long-term responses reported here were in contrast to those observed in lethal doses of BKME where ventilation volume, oxygen uptake, buccal pressures, and cough frequency rise to high levels and remain elevated for some hours before death.

Arterial Oxygen Tension Measurements

Arterial PO_2 recorded from sockeye in clean water ranged between 80 and 124 mm Hg. After exposure to KME arterial tensions ranged from 17 to 74 mm PO_2 and remained consistently below pre-exposure levels for up to 26 hr (Fig. 5). Data from a 150 g rainbow trout and 4-year-old sockeye adult in full and semibleach effluent respectively, showed similar results.

The oxygen dissociation curve prepared for sockeye salmon blood (Fig. 6) illustrates that pre-exposure arterial tensions are associated with 90–95% oxygen saturation of the blood while exposure to effluent was accompanied by a drop in the percent saturation of the blood with most samples being 55–80% saturated. This depression was not related to a change in environmental oxygen supply since airstones maintained oxygen at full saturation levels. It should be emphasized that the blood curve in Fig. 6 was done in the absence of CO_2. In the physiological range of PCO_2 for salmonids of 2–3 mm Hg (Holeton and Randall 1967) the curve would be shifted to the right and the percentage saturation of the blood at a given PO_2 would be even lower (Cameron 1971).

Reduction in arterial O_2 tension could be related to several factors: depressed ventilation or circulation: gas exchange difficulties at the respiratory epithelium or some affect on the oxygen-carrying capacity of the blood. Depression of ventilation certainly did not occur on initial exposure to KME, in fact, $\dot{V}_G$ and $\dot{V}_{O_2}$ increased. Efficiency of gas exchange may have suffered, due to abnormal breathing patterns, as described earlier, lowering the arterial tension. Another factor may have been an increase in the diffusion distance for gas exchange at the gills. The thickness of the respiratory epithelium is between 1 and 5μ (Newstead 1967) but mucus elaboration may increase the diffusion distance between blood and water. Walden and Howard (1968) reported excessive mucus production at the gills of salmon in neutralized BKME concentrations above 2–5% of full strength. We have observed mucus streaming from the gills of young coho in lethal doses of BKME.

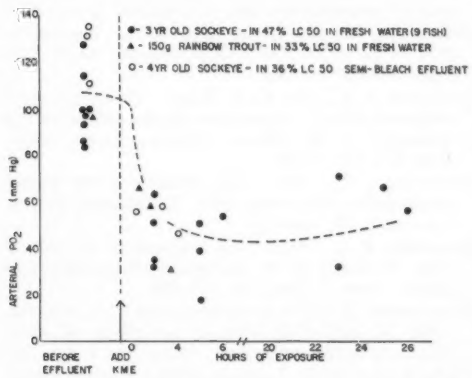

FIG. 5. Arterial oxygen tensions recorded from dorsal aortic blood samples from sockeye salmon and rainbow trout before and after KME addition. Data are individual readings and full-bleach kraft mill waste was used for the trout and 3-year-old sockeye.

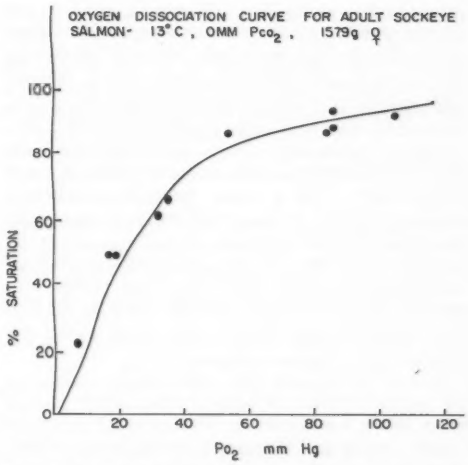

FIG. 6. A blood curve prepared for adult sockeye salmon blood. Values are individual readings obtained from specially-mixed samples of saturated and unsaturated blood.

Conclusions

These experiments have demonstrated that sublethal concentrations of filtered, aerated, neutralized BKME caused alterations in ventilatory function and reduced arterial oxygen tension in salmon. These results support the observations of Alderdice and Brett (1957) and Walden and Howard (1968) who observed "respiratory stress" in salmon in kraft mill effluent. It is reasonable to deduce that respiratory alterations such as those described here might interfere with the oxygen supply for

metabolism, particularly during activity when oxygen demand is high. Howard (unpublished data) demonstrated reduced mean critical swimming speed in trained juvenile coho salmon in neutralized, filtered BKME in a stamina testing device. The lowest concentration which significantly impaired swimming was 0.2 TL_m96 — the same sublethal threshold for the ventilatory changes reported here. It seems highly likely that a toxic mechanism affecting swimming ability in BKME is related to ventilatory abnormality and reduced oxygen saturation of arterial blood. These findings are in general agreement with those of Webb and Brett (1972) who reported reduced specific growth rates in 10 and 25% BKME (22 and 54% of the 4-day LC50) for underyearling sockeye salmon at 15 C.

It must be stressed that these results deal with the sublethal effects of aerated, neutralized, filtered BKME which has probably lost most of its more volatile toxicants. Volatile toxicants likely disappear rapidly in well-mixed receiving waters, however, toxicity may remain due to persistent chemicals plus the detrimental effects of *p*H, suspended fiber, and high BOD if the effluent is untreated. A great deal more work is required to identify the toxic constituents in pulp mill wastes and develop sensitive sublethal tests procedures for toxicity evaluation.

Acknowledgments

I am grateful for the cooperation of Canadian Forest Products Limited, Howe Sound Division. Mr C. Bulger and his staff provided assistance in effluent collections and regularly reported on operating characteristics of the mill. The technical assistance of Miss B. Mason and Mr I. Shand is greatly appreciated.

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Metabolic Rates and Critical Swimming Speeds of Sockeye Salmon (*Oncorhynchus nerka*) in Relation to Size and Temperature

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BRETT, J. R., AND N. R. GLASS. 1973. Metabolic rates and critical swimming speeds of sockeye salmon (*Oncorhynchus nerka*) in relation to size and temperature. J. Fish. Res. Board Can. 30: 379–387.

Ten years research on metabolic rates and swimming speeds of sockeye salmon (*Oncorhynchus nerka*) ranging in weight from 2 to 2000 g, at temperatures from 2 to 24 C, is reviewed and summarized. Analysis of weight–slope relations (*b* values) at three temperatures, using log–log transformations, provided an overall mean of 0.88 for standard metabolism and 0.99 for active metabolism. A previously determined semilog equation for temperature effect on standard metabolic rate (at approximately 50 g) was supported by supplementary data at 2 C. Predictive graphic models in the form of isopleths of metabolic rates and critical swimming speeds in relation to weight, length, and temperature are depicted. These provide a composite presentation useful in estimating the metabolic rate and maximum sustained speed for any size and temperature.

BRETT, J. R., AND N. R. GLASS. 1973. Metabolic rates and critical swimming speeds of sockeye salmon (*Oncorhynchus nerka*) in relation to size and temperature. J. Fish. Res. Board Can. 30: 379–387.

Les auteurs passent en revue et résument les résultats de dix années de recherches sur les taux métaboliques et les vitesses de nage du saumon nerka (*Oncorhynchus nerka*) de poids allant de 2 à 2000 g, et sur une gamme de températures de 2 à 24 C. L'analyse des relations poids–pente de courbe (valeurs de *b*) à trois températures, à l'aide de transformations log–log, donne une moyenne générale de 0.88 pour le métabolisme basal et 0.99 pour le métabolisme au cours de l'activité. Les données supplémentaires à 2 C corroborent une équation semi-logarithmique déjà établie en rapport avec l'effet de la température sur le taux de métabolisme basal (pour des sujets d'environ 50 g). Les auteurs décrivent des modèles graphiques permettant de faire des prévisions et ayant la forme d'isoplèthes des taux métaboliques et des vitesses critiques de nage en rapport avec le poids, la longueur, et la température. Ces modèles fournissent un ensemble utile pour l'estimation du taux métabolique et de la vitesse maximale de nage soutenue, à toutes taille et température.

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IN 1957 a proposal was made to build a series of hydroelectric dams on the Fraser and Thompson rivers[1], two of the best salmon producing rivers in British Columbia. Among the many possible consequences to salmon were the effects of elevated temperature and altered river flows on energy expenditure during migration. To better interpret and predict the consequences, a research plan was formulated in 1961 to determine the metabolic rate accompanying all levels of sustained performance at all tolerable temperatures for any size of sockeye salmon (*Oncorhynchus nerka*), the most valuable of the five species of indigenous Pacific salmon. Various stages in the development of this research have been reported, commencing with an outline of the general effects of environmental factors on respiratory metabolism of salmon (Brett 1962), followed by an account of the design and application of a tunnel respirometer (Brett 1964), the relation of size to metabolic rate and

[1]Although none of the original plans was finally accepted following extensive study, alternative proposals for electric power are still under consideration.

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performance of sockeye salmon at one temperature (15 C, Brett 1965a), and the relation of performance to temperature for one size (15 ± 3 cm, Brett 1967).

Further studies have been conducted, mostly at 5 and 20 C, but also a few at 2 C, with some supplementary data obtained at 15 C. It is the purpose of the following account to finalize the reporting by assembling the balance of information on metabolic rates and to present a composite picture of the size × temperature × speed relations.

The effect of size on resting metabolic rates of animals has occupied considerable attention. The general relation is usually described by the allometric equation $Y = aX^b$, where Y = rate of oxygen consumption and X = body weight. The rate per unit weight usually diminishes with increasing size both within and among species such that, for instance, in sockeye salmon the standard metabolic rate of a 3000-g adult is about one-fifth that of a 1-g fry, or, in the popular elephant–mouse example, the fraction is more like 1/50th. The need for accurate estimates of rate of change of metabolism with size (weight exponent *b*) has been well recognized. Recently, Glass (1969) has discussed the statistical limitations associated with deriving error estimates, pointing out a possible limitation in the log–log transformation so frequently used. An iterative, curve-fitting method was shown to be superior for data unevenly scattered over a wide range of weights. A reanalysis of the parameters *a* and *b* from published literature was performed. The disparity in mean slope values for all cases calculated was: $b = 0.8803$ (log–log transformation), compared with $b = 0.9832$ (iterative least squares). Further analysis of the sockeye data along similar lines has been conducted and is presented in this account.

Materials and Methods

At most temperatures a range of weights between 2 and 2000 g was used. This involved four distinguishable growth stages: fry, which are freshwater dependent; fingerlings, which have transformed into a saltwater tolerant stage (presmolt to smolt); jacks, which are small, precocious males; and adults, which are 4- and 5-year-old maturing fish. The fry and fingerling stages, with some intermediate sizes, were all cultured from eggs of wildstock parents; the jacks and adult salmon were trapped in June and July while migrating up the Stamp River, Vancouver Island, B.C.

Approximately 10 days of acclimation for every 5 C difference from ambient temperature was allowed for thermal adjustment. Temperature was controlled to within ±0.3 C in the holding tanks and ±0.2 C in the respirometer.

Almost every period of the year was involved during testing, with the months of June–September predominating, particularly in the case of high acclimation temperatures (20 C and above). No separation of season, sex, age, stage, and maturity effects was attempted in the analysis, for insufficient data were available. These factors undoubtedly account for a fair portion of the variability represented by the error estimates (see Table 2).

Two respirometers were used, one with a minimum volume of 13.0 liters (Mar 1959; Brett 1964) and the other with a minimum volume of 110 liters (Bell and Terhune 1970). In addition, adult salmon were tested for swimming speed in a 28-cm diameter tunnel (Fig. 1). For fish under 10 g in weight the removal of oxygen from the small respirometer was insufficient in 1 hr to allow for accurate estimates of rate of oxygen consumption, mostly determined by serial Winkler titrations. It was therefore necessary to use a group of from three to five fish in the chamber. In these instances, calculations were based on mean weights and lengths of each group, and considered as a single statistic.

A standard, stepwise increase in tunnel velocity at 1-hr intervals has been used throughout all experiments. The development of this incremental procedure, which takes the fish in stages up to a fatigue velocity (critical swimming speed), has been discussed in Brett 1964 and 1967. The oxygen consumption rates are plotted as the logarithm of uptake (mg O_2/kg/hr) against velocity (cm/sec or length [L]/sec), which linearizes the relation under conditions of steady performance. Velocities below 0.5–1.0 L/sec were found to be useless because metabolism from spontaneous activity was usually in excess of that from low imposed velocities. Standard metabolic rates were determined by extrapolation of the linearized relation to zero velocity i.e. complete rest.

For larger fish, which occupy a substantial area of the cross section of the water tunnel, the true speed relative to flow-rate was estimated by recording tailbeat frequency. It has been shown that a direct relation exists between tailbeat frequency and speed for all but the lowest velocities (Bainbridge 1958). This was confirmed and applied to the sockeye salmon to determine the equivalent swimming speed for fish in excess of 30 cm (approximately 300 g).

Results

Weight Relation

The data relating active and standard oxygen consumption rates to weight, at three temperatures, are presented in Fig. 2, plotted on log–log axes.

Following the recommendation of Glass (1969, 1970) to test the validity of performing a logarithmic transformation of metabolic rate data and subsequent linear regression, Bartlett's test for homogeneity of variance was computed for a comparison of *k* sample variances (s_i^2), one for each weight class within each temperature (each

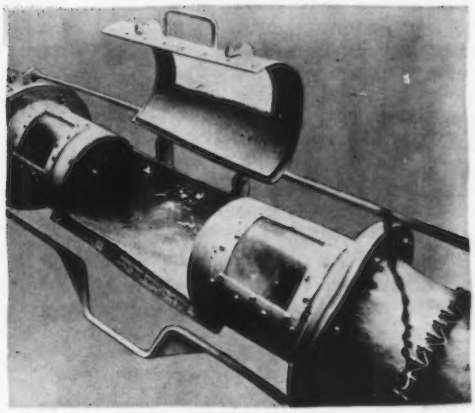

FIG. 1. Photograph of the fish chamber section of a steel pipe used for determining maximum sustained swimming speeds of adult sockeye salmon (*Oncorhynchus nerka*). The diameter is 28 cm (11 inches); a meter rule (39 inches) is shown below the open hatch. An electric charge (usually 5–10 v, 60 c, a-c) was applied to the downstream aluminum vanes imbedded in a fiberglass section (bottom right). Water velocity of 2.8 m/sec (11 ft/sec) could be obtained.

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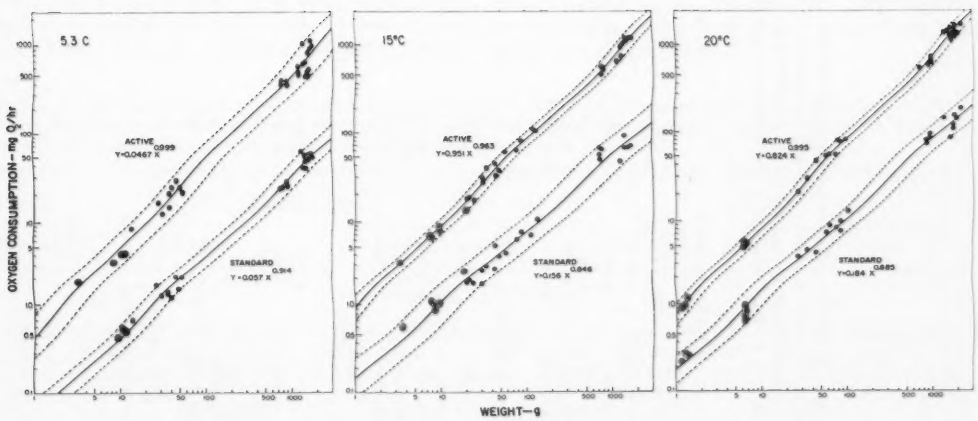

FIG. 2. The relation between metabolic rate and weight for sockeye salmon acclimated to and tested at 5.3, 15, and 20 C. Circled points indicate cases where groups of three to five fish were used together. Broken lines represent the 95% confidence limits for individual values, determined by least-squares of the logarithms. Table 2 shows confidence limits of derived parameters.

TABLE 1. Results of Bartlett's test for homogeneity of variance. Data are given by temperature, weight class, and for raw and logarithmically transformed standard and active metabolic rate data from sockeye salmon (*Oncorhynchus nerka*). The number of observations (n) is generally equal to the number of fish tested. In those instances where the number of fish per observation is greater than one, an asterisk appears in the weight class column. ξ indicates homogeneous variance at the 5% level of significance for the appropriate df.

Temp	Wt class (g)	Mean standard metabolism (mg O_2/hr)	s^2	Mean log standard metabolism	s^2	n	Mean Active metabolism (mg O_2/hr)	s^2	Mean log active metabolism	s^2	n
5.3 C	5–20*	0.562	0.010	–0.255	0.006	4	4.905	5.338	0.660	0.032	4
	20–60	1.540	0.128	0.178	0.009	8	19.378	23.508	1.275	0.013	8
	700–900	23.650	1.957	1.374	0.001	4	401.500	595.000	2.603	0.001	4
	1000–2000	48.155	44.269	1.679	0.004	11	703.308	49046.897	2.815	0.019	14
	df = 3			df = 3			df = 3			df = 3	
	$\chi^2 = 51.62$			$\chi^2 = 3.74^{\xi}$			$\chi^2 = 69.29$			$\chi^2 = 6.15^{\xi}$	
15.0 C	5–19*	0.980	0.007	–0.019	0.001	4	7.587	1.416	0.877	0.004	3
	19–60	2.501	0.734	0.377	0.020	10	27.600	117.909	1.409	0.033	10
	70–150	7.976	3.498	0.891	0.010	5	88.860	461.858	1.937	0.013	5
	700–800	53.425	47.989	1.725	0.003	4	562.750	3272.917	2.749	0.002	4
	1000–2000	65.560	288.233	1.805	0.013	5	1006.778	31631.194	2.996	0.007	9
	df = 4			df = 4			df = 4			df = 4	
	$\chi^2 = 69.82$			$\chi^2 = 7.32^{\xi}$			$\chi^2 = 66.40$			$\chi^2 = 9.83$	
20.0 C	1–5*	0.266	0.001	–0.577	0.002	3	1.01	0.012	0.003	0.002	3
	5–7*	0.808	0.013	–0.096	0.004	4	5.25	0.151	0.720	0.001	5
	20–50	4.09	0.152	0.611	0.002	3	31.0	137.843	1.472	0.027	3
	50–100	9.08	4.487	0.939	0.011	6	64.3	213.851	1.799	0.010	6
	800–1000	87.0	162.0	1.937	0.004	2	629.7	1600.333	2.798	0.001	3
	1000–2200	137.4	1321.62	2.124	0.015	7	1364.0	16236.544	3.133	0.002	10
	df = 5			df = 5			df = 5			df = 5	
	$\chi^2 = 76.49$			$\chi^2 = 4.21^{\xi}$			$\chi^2 = 77.20$			$\chi^2 = 13.38$	

with f_i df). For this test, the following statistic was calculated:

$$\chi^2 = \frac{(\sum f_i)\ ln(s^{-2}) - \sum[f_i\ ln(s_i^2)]}{1 + \frac{1}{3(k-1)}\left[\sum\frac{1}{f_i} - \frac{1}{\sum f_i}\right]}$$

where $s^{-2} = \frac{\sum f_i\ s_i^2}{\sum f_i}$, and summations are over k; s^{-2} is approximately normally distributed according to the χ^2 distribution with k-1 df. It tests the null hypothesis that each of the s_i^2 is an estimate of the same population σ^2. Bartlett's test was performed on the raw data and on the data after they had been transformed by means of logarithms (Table 1).

For each of the three temperatures, all weight groups exhibit homogeneous variances for the logarithmically transformed data on standard metabolism. For untransformed standard metabolic rate data, variance is not homogeneous and is a progressively increasing function of body weight. This indicates that a log transformation for these data is in order and that linear least-squares regression to determine parameter values is appropriate.

The conclusion for the active metabolic rate data is not so straightforward. For untransformed data the variance is not homogeneous (again it is a progressively increasing function of body weight). However, for logarithmically transformed active metabolism data, homogeneity of variance appears to hold for the 5.3 C tests. For data taken at 15.0 and 20.0 C, χ^2 is too large and homogeneity cannot be adequately demonstrated. An examination of variances for these two temperatures indicates that the variances differ from each other in a random fashion. Therefore, increasing the sample size within each weight group or increasing the degrees of freedom by more individuals in more size classes, or both, might result in an acceptably small value for χ^2. In the absence of additional data for active metabolic rate, these data can be tentatively assumed to have homogeneous variance on the grounds that the variances appear to be random, and on the grounds that the computed χ^2 is not greatly larger than that required for the 5% level of significance.

A comparison of the parameters a (intercept) and b (slope) computed as log–log transformations appears in Table 2. Since the distribution of the data points does not suffer from a comparative lack of representation of large weights in relation to small weights, the equations derived for "log–log" least squares have been used in subsequent calculations (e.g. Fig. 3), mostly without extrapolation beyond the range of weights tested.

Temperature Relation

The metabolic rates at 2 C for five fish of mean weight 42.1 g (length 16.0 cm) are recorded in Table 3. The predicted standard metabolic rate per unit weight from the equation[2] derived earlier (Brett 1964) was 40.2-mg O_2/kg/hr. This compares

[2]For the size in question, $\log(Y - 36) = 0.48 + 0.071X$, where Y = oxygen consumption in mg O_2/kg/hr and X = temperature (C).

Table 2. Parameter estimates of the fitted curves for *oxygen consumption–body weight* relations described by the power function $Y = aX^b$. The mean values and 95% confidence limits are compared when derived by the method of least squares (log–log) and by iterative curve fitting (nonlinear least squares).

	a (*intercept*)		*b* (*slope*)	
Temp (*C*)	log–log	nonlinear	log–log	nonlinear
		Standard Metabolism		
5.3	0.0181<0.0565<0.0949	0.0264<0.0282<0.0300	0.8988<0.9141<0.9294	0.997<1.014<1.022
15.0	0.1008<0.1498<0.1988	0.428 <0.484 <0.540	0.8233<0.8465<0.8697	0.658<0.682<0.696
20.0	0.1229<0.1987<0.2745	0.240 <0.270 <0.299	0.8505<0.8844<0.9183	0.807<0.834<0.845
Avg	–	–	0.8817	0.8433
		Active Metabolism		
5.3	0.4157<0.4667<0.5177	0.338 <0.388 <0.439	0.9787<0.9989<1.0191	0.969<1.027<1.043
15.0	0.9288<0.9513<0.9738	0.724 <0.753 <0.781	0.9534<0.9632<0.9730	0.989<0.996<1.001
20.0	0.8062<0.8237<0.8412	1.682 <1.772 <1.862	0.9872<0.9947<1.0022	0.879<0.888<0.895
Avg	–	–	0.9856	0.9703

favorably with the observed rate of 42.2 ± 2.4 SE mg O_2/kg/hr.

The active metabolic rate of 286 ± 28.4 SE mg O_2/kg/hr was considerably below the predicted rate of approximately 460-mg O_2/kg/hr, derived graphically (see Fig. 13 in Brett 1964 and Fig. 2 (20 C) in this paper). The critical swimming speed (40 ± 2 cm/sec, 2.5 L/sec) that accompanied the observed metabolic rate was close to that expected (41 cm/sec, 2.6 L/sec — see Fig. 4). This apparently efficient but atypical metabolic rate defies immediate explanation. It is possible that seasonal effects coupled with the small sample produced results at such variance from the expected. It stands as one of the few divergent cases among many supporting data in the overall study.

Speed Relation

From the previous records published on maximum 60-min sustained swimming speeds (Brett 1964, 1965a, and 1967), and the additional records obtained (Table 4), the following set of equations were derived for each of the temperatures indicated:

2 C	$\log Y = 0.9053 + 0.6294 \log X$
5 C	$\log Y = 0.9555 + 0.6243 \log X$
10 C	$\log Y = 1.0346 + 0.6294 \log X$
15 C[3]	$\log Y = 1.1289 + 0.6345 \log X$
20 C	$\log Y = 1.1031 + 0.6293 \log X$

where Y = speed (cm/sec) and X = total length (cm). The data were too scant at 2 and 10 C to formulate independent equations so a mean slope, passing through the geometric mean position (log scale) of X and Y for each of the above two temperatures, was applied from the other determinations. These mean values correspond to the loci of speed in relation to temperature, as depicted in Fig. 21 in Brett 1964. The derivation assumes that the rate of change of critical speed with length is not temperature dependent i.e. that the slopes do not differ with temperature. In support of this assumption can be cited the fact that active metabolic rate, induced by critical swimming speed, is nearly size independent at all temperatures (see *b* values for active metabolism in Table 2).

Length-Weight Relation

Since the propulsive force in swimming is derived from the length of the body to the tip of the tail, total length is usually recorded for use in performance records. The equation relating weight in grams (X) to total length in centimeters (Y) for the whole sample was:

$$\log Y = 0.7066 + 0.3187 \log X$$

This equation has been used for relating length and weight for the speed graph in Fig. 4.

Discussion

As stated, considerable attention has been devoted to examining the weight exponent (*b* value) of metabolic relations, concentrating on the possible physiological significance of surface or weight proportionality for standard metabolic rates. Because of the great differences in values, ranging from 0.5 to 1.0 (Fry 1971), and the possibility that the best fit was not always determined, this path of enquiry has not been particularly profitable for either understanding the process or providing a broad predictive model.

Although the value of *b* (log–log) for standard metabolism at 5.3 C differed significantly from that at 15 C, neither differed significantly from the 20 C value (Table 2). The mean of all three was 0.88. By the iterative, least-squares regression procedure a significant difference occurred between all standard values, but no consistent temperature trend appeared; the mean value was 0.84.

With regard to active metabolism, for both forms of calculation the average *b* value at all temperatures[4] so closely approximates 1.0 that both weight and temperature independence can be ascribed to the slope relation for active rates.

Since fish are usually subject to daily and seasonal changes in environmental temperature, activity, and feeding, it is only possible to conceive of very transitory and limited periods during which the actual "field" metabolic rate could be estimated with any accuracy by applying the equations for standard or active metabolic rate for a known weight. However, the estimates do provide the limits within which the well-fed fish is functioning. Some measure of daily activity coupled with documented thermal experience is necessary for improving the accuracy of estimates for use in any ecological context. In this respect an effort was made to compute the hourly metabolic rate of lake-dwelling sockeye fingerlings (2 g) in midsummer, when they experience temperature changes of 10 C during daily vertical migrations, accompanied by surface feeding (Brett 1971). The estimated

[3]Data for 15 C corrected for tunnel interference from those published in Brett (1965b).

[4]With the exception of one determination at 20 C nonlinear, Table 2.

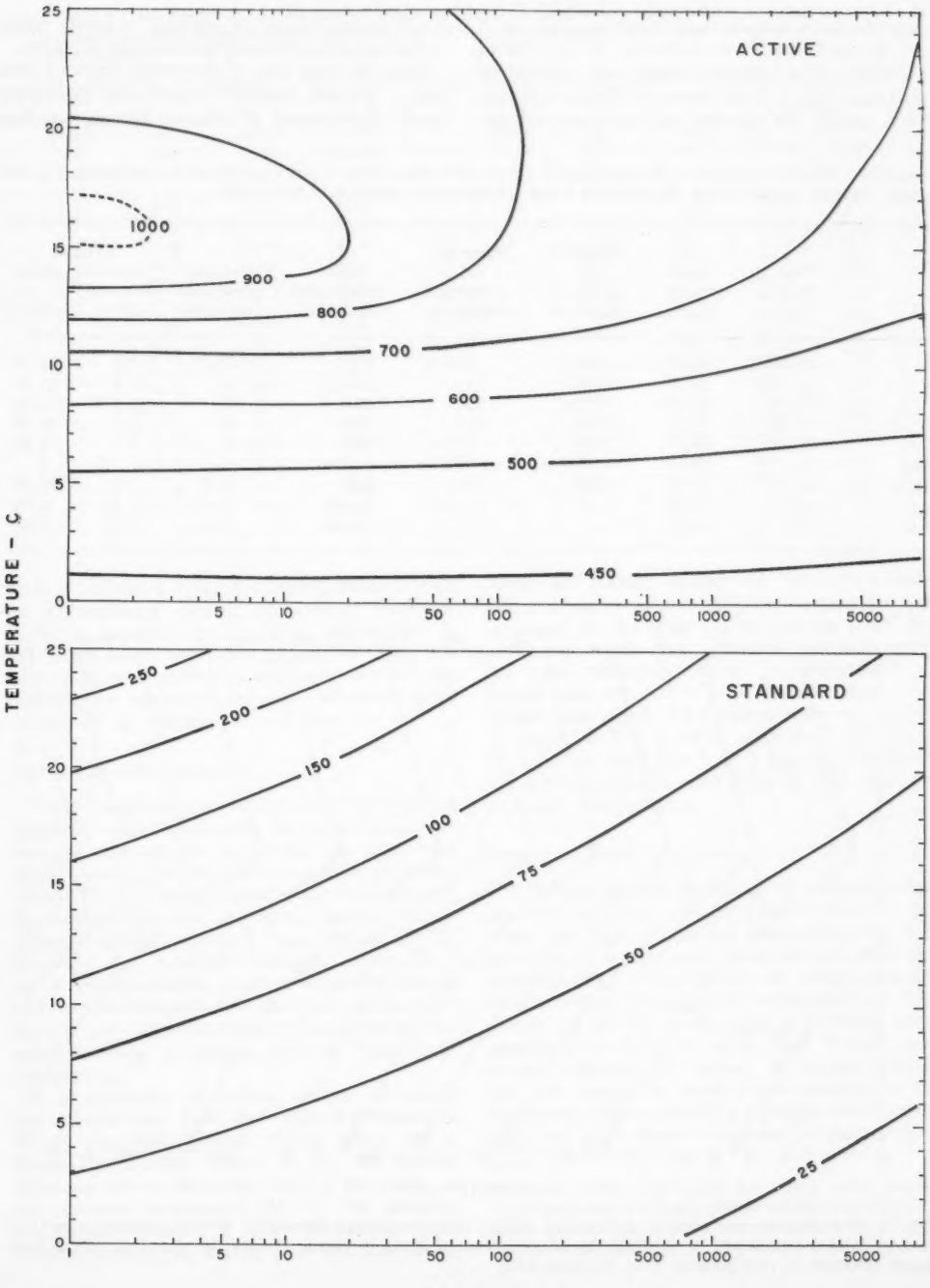

FIG. 3. Temperature–weight response surfaces for active and standard metabolic rates of sockeye salmon represented by isopleths for selected rates of oxygen consumption in mg O_2/kg/hr. Dotted line for 1000-mg O_2/kg/hr obtained by extrapolation.

possible limits in rate were from standard (at 7 C), 60-mg O_2/kg/hr, to active (at 17 C), 950-mg O_2/kg/hr. The deduced range was 65–350-mg O_2/kg/hr, with a daily mean of 170-mg O_2/kg/hr. Thus, neither the standard nor active rate as predicted for the weight in question is within 200% of the deduced daily mean metabolic rate in nature.

Since the basic aim of the studies reported here was to provide predictive models for estimating energy expenditures of sockeye salmon, together

TABLE 3. Metabolic rate (per unit weight) and critical swimming speed of yearling sockeye acclimated to 2 C and tested between August 25 and September 9. Rates determined as described in Brett 1964.

	Wet weight (*g*)	Total length (*cm*)	Maximum O_2 consumed (*mg/kg/hr*)	Minimum O_2 consumed (*mg/kg/hr*)	Active metabolism (*mg/kg/hr*)	Standard metabolism (*mg/kg/hr*)	Critical swimming speed (*L/sec*)	(*cm/sec*)
	49.04	16.4	171	69	175	47.2	2.9	34.44
	42.36	16.4	324	113	325	35	2.8	45.92
	39.80	15.7	318	130	320	46	2.4	37.68
	42.22	15.6	317	112	320	49	2.8	43.68
	36.95	15.9	290	95	290	41	2.4	38.16
No.	5	5	5	5	5	5	5	5
Mean	42.07	16.0	284	104	286	42.2	2.5	39.98
S.D.	4.47	0.38	–	–	63.58	5.45	0.30	4.69
S.E.	2.01	0.17	–	–	28.44	2.44	0.13	2.09

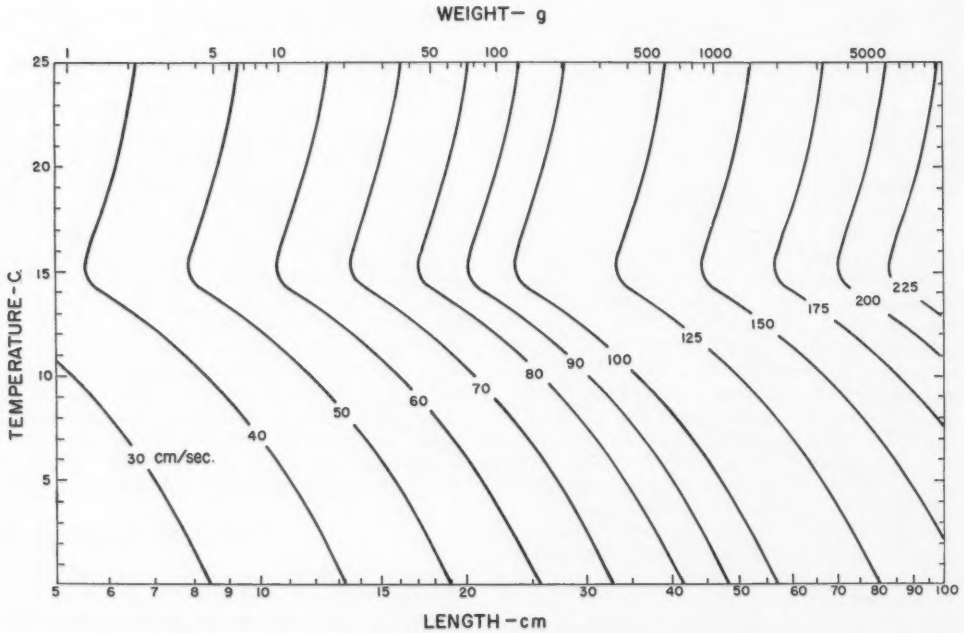

FIG. 4. Temperature–size response surface for critical swimming speed (maximum 60-min sustained speed) of sockeye salmon represented by isopleths for selected rates of speed in cm/sec. Values for sizes over 65-cm total length obtained by extrapolation from the basic data.

TABLE 4. Maximum 60-min sustained swimming speeds (critical speeds) determined in tunnel respirometers. Data for sockeye over 30 cm (300 g) corrected for restricting effect of tunnel by reference to open flume results.

Temp (C)	Wt (g)	Length (cm)	Speed (cm/sec)	Speed (L/sec)	Temp (C)	Wt (g)	Length (cm)	Speed (cm/sec)	Speed (L/sec)
5	8.9	9.2	40.5	4.4	20	5.6	8.6	55.6	6.5
"	10.7	9.9	40.8	4.1	"	5.6	8.8	55.0	6.3
"	13.6	10.9	41.5	3.8	"	5.6	8.1	53.9	6.7
"	36.7	16.6	54.2	3.3	"	5.8	8.7	52.9	6.1
10	32.8	16.2	59.0	3.7	"	6.1	9.4	64.4	6.9
15	3.4	7.7	51.5	6.7	"	6.3	9.0	61.9	6.9
"	8.5	10.0	59.8	5.9	"	7.4	9.8	57.6	5.9
"	19.1	12.8	53.2	4.2	"	7.7	9.8	62.5	6.4
"	55.2	18.8	90.7	4.8	"	1385.0	53.0	127	2.4
"	746.0	41.8	150	3.6	"	1530.0	57.0	127	2.2
"	1432.0	53.9	178	3.3	"	1611.0	56.5	134	2.4
20	1.1	5.6	39.7	7.1	"	1688.0	55.3	129	2.3
"	1.2	5.8	37.1	6.4	"	1784.0	56.0	126	2.3
"	1.3	5.5	45.1	8.2	"	1794.0	59.5	136	2.3
"	1.4	5.9	43.1	7.3	"	1962.0	61.4	128	2.1
"	1.7	6.1	46.0	7.6	24	52.2	18.5	69.4	3.8

with an informed opinion of the possible impact of environmental change, an attempt has been made to assemble the available information in the most direct way for application. This has been done in the following manner by constructing isopleths for metabolic rate and swimming speed in relation to temperature and size.

METABOLIC RATE ISOPLETHS

Values from the equations for active and standard metabolic rates in relation to weight and temperature were plotted in arrays, and lines were drawn through the interpolated loci for particular values (Fig. 3). Some extrapolation was necessary for weights below 2 g and above 2000 g. The resulting isopleths provide a rapid means for determining the maximum metabolic rates (associated with maximum sustained swimming speeds) and the minimum rates (for resting or zero activity) for any size and any tolerable temperature. The graph provides a suitable sort of "handbook" presentation.

It is interesting to note a number of aspects that become clear from this form of compilation. Weight has least influence on the active rate at lowest temperatures (below 10 C); the greatest difference occurs along the ridge in the region of the optimum temperature (15 C). The optimum temperature produces a more pronounced maximum metabolic rate among small fish than among large; the limiting factor for respiratory–circulatory capacity (Jones 1971) is apparently not expressed to the same extent among large fish as among small ones. Standard metabolic rates are quite differently related to temperature × weight than are active rates; greater relative extremes exist within the standard rates e.g. 250-mg O_2/kg/hr for 2 g at 24 C compared with 25-mg O_2/kg/hr for 2000 g at 3 C, a factor of 10 times; the active metabolic rates differ by 3.5 times for the same circumstances.

SWIMMING SPEED ISOPLETHS

A similar process of setting up arrayed values was used to obtain the isopleths of Fig. 4. Once again the ridge of highest performance for any given length is generated along the optimum temperature axis (15 C). Because of assumptions on slope relations (temperature independent), a uniformity of pattern results; this is probably oversimplified.[5] It is worth noting that, if sizes were scaled arithmetically instead of logarithmically, the lines would be much more extended in the horizontal plane; a doubling of length has a greater effect on speed than a doubling of temperature.

[5]Glova (1972) has found for young coho salmon (*O. kisutch*) that the slopes of the lines relating swimming speed to length (at a given temperature) increased with elevated temperature.

At 15 C a 5-cm fish can swim 7 L/sec, whereas a 50-cm fish can only achieve 3 L/sec.

Intermediate Rates

From the three sets of isopleths it is an easy matter to obtain estimates for the metabolic rate accompanying any level of sustained locomotion. This is possible because of the simple geometric relation between power (metabolic rate) and performance (speed). An example will suffice. Suppose a 4-g, 8-cm smolt, swimming at half its critical speed, entered an environment in which the temperature was elevated by 5 C (from 10 to 15 C). Using semilog paper (Fig. 5), vertical lines are erected at the maximum sustained speeds (obtained from Fig. 4) for this size fish at 10 (40 cm/sec) and 15 C (48 cm/sec). The active and standard metabolic rates (from Fig. 3) are then marked appropriately and joined by a straight line for each temperature (exponential transformation). The consequent increased energy demand may now be estimated: case 1 (remaining at the same speed of 20 cm/sec in the new environment), the metabolic rate would rise from 210- to 280-mg O_2/kg/hr, an elevation of 33%; case 2 (increasing to one-half the new critical speed i.e. 24 cm/sec), the rate would rise from 210- to 330-mg O_2/kg/hr, an elevation of 57%.

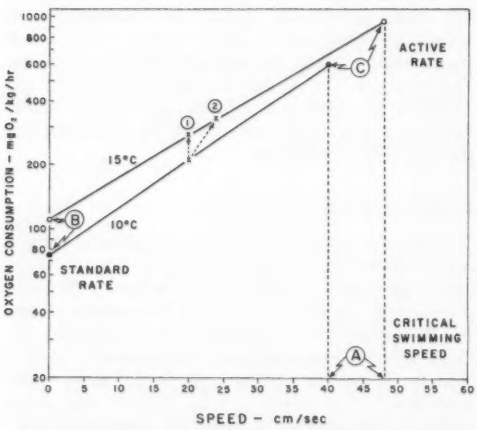

FIG. 5. Relation of metabolic rate to swimming speed at 10 and 15 C determined graphically for a 5-g, 8.5-cm sockeye smolt. Position of vertical lines at A for critical swimming speeds at the chosen temperatures obtained from Fig. 4; metabolic rates indicated by points at B and C obtained from Fig. 3. The expected change in metabolic rate induced by a 5 C increase in temperature, when swimming at half the critical speed, is indicated for two possible cases at positions 1 and 2.

This example represents the sort of impact that a large reservoir above a dam would have on downstream migrants. The increased energy expenditure would probably lie between the two estimates, at 45% increase.

Concluding Comment

The active metabolic rate of sockeye salmon is one of the highest on record, exceeding that determined for other salmonids by 30-40% (Fry 1957; Altman and Dittmer 1968). From a resting rate of 50-mg O_2/kg/hr in midwinter under ice-cover (4 C) to an excited metabolic state of 340-mg O_2/kg/hr while feeding near the surface in midsummer (17 C), it is estimated that the average metabolic rate of a fingerling (2 g) would differ by a factor of 7 times (Brett 1971); the average metabolic rate of an adult sockeye migrating up the Fraser River in midsummer would approximate 550-mg O_2/kg/hr or 45 cal/kg/day (Brett 1965b).

These are well-founded predictions indicating the present state of understanding of rates of energy expenditure for sockeye salmon. By examining one species in depth and developing suitable methodology, it should now be possible to arrive at a comparable state of knowledge for other species of Pacific salmon in a relatively small fraction of the time and effort that was necessary for this first species.

Acknowledgments

This lengthy study has depended on the technical support of a number of dedicated assistants. In particular, Mr D. B. Sutherland and Mr C. T. Shoop have faithfully carried out numerous, onerous tasks. Dr J. C. Davis (then a student) conducted much of the testing of adult sockeye in the large respirometer.

The analysis of size effects was sufficiently benefited by the critical insight of Dr N. R. Glass that a combined authorship was generated through his association. It would not be in keeping, however, to attach responsibility for the collecting of the data to him; this aspect is shared by the above assistants with J. R. Brett.

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A New Species of Marine Triclad Turbellarian of the Genus *Sabussowia* from Prince Edward Island

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BALL, I. R. 1973. A new species of marine triclad turbellarian of the genus *Sabussowia* from Prince Edward Island. J. Fish. Res. Board Can. 30: 389–394.

A new species of marine triclad turbellarian, *Sabussowia wilhelmii* sp. nov., is described from the Pisquid River, Prince Edward Island, Canada. This represents the first occurrence of the genus and of the subfamily Cercyrinae of the Procerodidae in North America. The genus is discussed and a key to the known species is provided. A detailed discussion of the status of *Vortex warreni* of Girard and *Procerodes warreni* of Wilhelmi is given. The identity of the former cannot be ascertained. The latter could be a *Sabussowia* species, but, in the absence of fully mature material, this question cannot be resolved. Both names are regarded as nomina dubia, and the suppression of the genus *Fovia* of Girard is recommended.

BALL, I. R. 1973. A new species of marine triclad turbellarian of the genus *Sabussowia* from Prince Edward Island. J. Fish. Res. Board Can. 30: 389–394.

L'auteur décrit une espèce nouvelle de turbellarié marin de l'ordre des triclades, *Sabussowia wilhelmii* sp. nov., provenant de la rivière Pisquid, Ile-du-Prince-Edouard, Canada. Il s'agit de la première mention du genre et de la sous-famille des Cercyrinae, famille des Procerodidae en Amérique du Nord. L'auteur passe le genre en revue et donne une clé des espèces connues. Il examine en détail le statut de *Vortex warreni* de Girard et de *Procerodes warreni* de Wilhelmi. Il ne peut vérifier l'identité de la première espèce, alors que la dernière peut être une espèce de *Sabussowia*, mais en l'absence de spécimens ayant atteint pleine maturité, il est impossible de trancher la question. Il considère les deux noms comme *nomina dubia*, et recommande l'abolition du genre *Fovia* de Girard.

Received September 25, 1972

HITHERTO the genus *Sabussowia* Böhmig, 1906, a genus of marine triclads of the family Procerodidae, subfamily Cercyrinae, has been known only from Europe. With the collection of two fully mature specimens of an undescribed species from Prince Edward Island, I am now able to record for the first time the occurrence of this genus and subfamily from the Atlantic Coast of North America.

The present specimens show some striking similarities with the species described, albeit incompletely, from Woods Hole, Nobska Point, and Boston Harbour, Massachusetts, by Wilhelmi (1909) under the name *Procerodes warreni* (Girard, 1850), which name is also used by Kato (1943), Hyman (1944), and Gosner (1971), presumably in the same sense. Probably the *Fovia affinis* of Miner (1950) refers also to the same species. In my opinion, however, the reasons for which will be discussed below, Wilhelmi (1909) singularly fails to establish beyond doubt that his species is the same as that so poorly described as *Vortex warreni* by Girard (1850a). Furthermore, Wilhelmi had no fully mature material and so could not give a complete description, and he remained unsure of its true generic position. Hyman (1944), too, failed to obtain sexually mature material and thus could not resolve the problem.

It is impossible, therefore, to ascertain beyond doubt if my specimens are the same as those described by Wilhelmi (1909), though I do suspect that they may be the same genus. Consequently, and considering the new generic record, I am describing my specimens as a species new to science, though naming it for Wilhelmi to acknowledge his important contributions to the study of the Maricola.

Materials and Methods

The two specimens collected were studied alive, photographed in color, killed in Steinmann's fluid, and fixed in 70% alcohol. Both specimens were sectioned sagitally at 8-μm intervals; the stains used were Mallory

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Heidenhain (Gurr 1963) and Phosphotungstic Acid Haematoxylin (Drury and Wallington 1967).

Family Procerodidae Diesing, 1862
Subfamily Cercyrinae Böhmig, 1906
Genus Sabussowia Böhmig, 1906 : 359

Penis pointed, without a stylet. Vasa deferentia unite to a common duct which enters the penis bulb. Bursa copulatrix behind the penis. Ovaries behind the brain; oviducts enter separately the sides of the bursal stalk, entally (proximally) to the shell glands. Testes numerous, dorsal, throughout the body-length. With well-developed preocellar intestinal duct. Type: *S. dioica* (Claparède, 1863). Distribution: Europe, Atlantic Coast of North America.

Sabussowia wilhelmii sp. nov.

MATERIAL EXAMINED

Holotype: One set of sagittal sections on two slides, Mallory-Heidenhain stained, in the invertebrate collections of the National Museum of Natural Sciences, Ottawa, as follows: NMC Cat. No. 203.

Paratype: One set of sagittal sections on two slides retained in my private collection.

TYPE LOCALITY:

Pisquid River on Highway 21, just west of Pisquid, Queen's County, P.E.I., Canada. Collected from algal fronds in brackish water on July 22, 1969, by I. R. Ball.

ETYMOLOGY

The species is named for Julius Wilhelmi to commemorate his distinguished work on the Maricola.

EXTERNAL FEATURES (FIG. 1A)

Maximum length of living animals 4.5 mm. Anterior end rounded, without auricles, with a slight constriction behind the eyes. Body margins smooth and parallel. Posterior end truncate. Two eyes, equidistant from each other and their respective body margins, set in unpigmented areas about 10% of the body length from the anterior margin. Dorsal surface pale olive-brown in color, with two darker longitudinal stripes delimiting a pale area in the midline. There is a further concentration of pigment just behind the eyes and one at the anterior margin. Ventral surface paler. Root of pharynx about halfway along the body, pharynx one-fifth of the body-length. Gonopore lies close behind the pharyngeal pore.

EPIDERMIS

The dorsal (ca. 8 μm) and ventral (ca. 9 μm) epithelia are clothed with short cilia and packed with rhabdites. They appear to be nucleate, but accurate determination is made difficult by the density of the rhabdites. Beneath the basement membrane on which the dorsal epithelium sits are three layers (< 2 μm) of muscle fibers, circular, diagonal, and longitudinal. The ventral musculature is similar but the circular (ca. 4 μm) and longitudinal (ca. 6 μm) layers are much thicker. Numerous dorso-

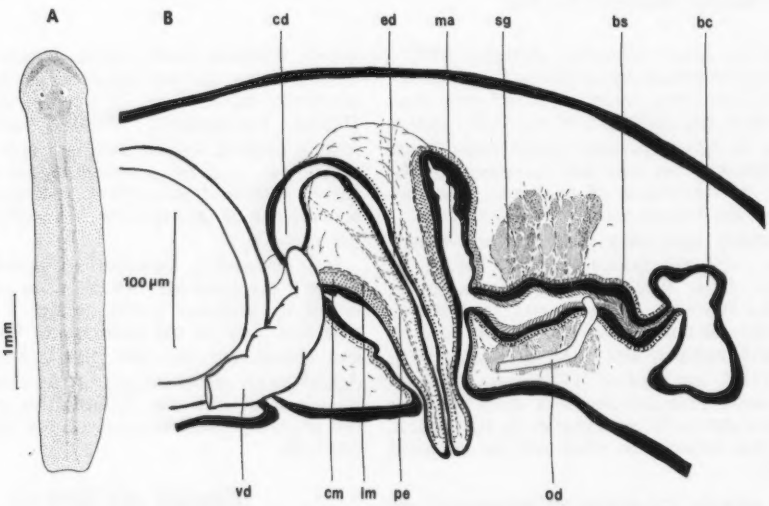

FIG. 1. A, *Sabussowia wilhelmii* sp. nov. External features of living animal. Drawn from a photograph; B, Holotype. Diagrammatic sagittal section of the copulatory apparatus, viewed from the left side: bc, bursa copulatrix; bs, bursal stalk; cd, common vas deferens; cm, circular muscle; ed, ejaculatory duct; lm, longitudinal muscle; ma, male atrium; od, left oviduct; pe, penis; sg, shell glands; vd, left vas deferens.

ventral fibers, derived from the longitudinal layers, traverse the parenchyma. Numerous pigment granules are distributed beneath the dorsal dermal musculature and, at the tip of the anterior end only, beneath the ventral dermal musculature. The marginal adhesive glands are not prominent.

Alimentary System

The anterior ramus of the intestine extends anteriorly between the eyes and forms two or three pairs of preocellar diverticula. The two posterior rami do not anastamose posteriorly. The pharynx is constructed as follows: outer epithelium with cilia, longitudinal muscles (ca. 1 μm), circular muscles (ca. 4 μm), parenchymatous zone containing nervous and glandular elements (66–85 μm), inner longitudinal muscles (1–4 μm), inner circular muscles (8–30 μm), and a ciliated inner epithelium. The musculature is weakest terminally. The pharyngeal pocket and intestinal lumen are infected with large numbers of gregarine parasites.

Female Reproductive System (Fig. 1B)

The paired ovaries are relatively large (ca. 115 × 96 μm), lobate, ovoid bodies situated above the ventral nerve cord, just behind the brain, about 650 μm from the anterior end. The oviducts leave the ventro-lateral face of the ovaries, curve posteriorly, and travel ventrally, far above and lateral to the large ventral nerve cords, to the level of the copulatory apparatus. Here they turn dorsad and mediad, and enter separately the lateral walls of the bursal stalk, closer to the bursa copulatrix than to the atrium. The oviducts are lined with a nucleate, ciliated epithelium. The bursa copulatrix is a sacciform organ, of irregular shape, situated behind the penis. From its frontal face the bursal stalk runs more or less horizontally to open into the posterior wall of the male atrium. The stalk is lined with a nucleate epithelium bearing long cilia, which is overlain by a thin muscle coat comprising an inner single layer of circular fibers and an outer single layer of longitudinal fibers. Extensive shell glands open into the bursal stalk distally to the oviducts, and this part is also considerably dilated. The epithelium of the bursa copulatrix consists of low vacuolated cells that are virtually indistingishable from those lining the intestinal lumen. The bursa does not possess a discernible muscle coat. The vitellaria are extensive, rich in inclusions, and occur dorsally and ventrally to the intestine, and sometimes ventrally to the oviducts. They extend from in front of the ovaries, to which some may be closely applied, to the posterior end of the animal.

Male Reproductive System (Fig. 1B)

The testes are numerous, of moderate size, and dorsally situated in the parenchyma throughout the body-length. The vasa deferentia are situated medially to the oviducts. At the level of the pharynx each vas deferens enlarges to form a false seminal vesicle, and posterior to these the now narrow vasa deferentia unite to a common extra-bulbar vesicle that opens into the ejaculatory duct, and this opens at the tip of the penis papilla. There is a dilation in the ejaculatory duct that forms an intrapenial seminal vesicle. The penis is large, and consists of a muscular hemispherical bulb embedded in the antero-dorsal wall of the atrium, and a conical, almost vertically orientated, papilla that tapers to a point. The basal half of the penis papilla is highly muscularized. Beneath the nucleate epithelium there is a thick layer of circular muscle fibers underlain by thin longitudinal fibers. The musculature weakens distally, and is absent at the very tip. The ejaculatory duct is lined with a nucleate epithelium covered with circular muscle fibers only. The male atrium is just spacious enough to accommodate the penis. Entally to the bursal stalk opening it has a papillate, nucleate epithelium underlain by a thick muscle layer of inner circular and outer longitudinal fibers. Elsewhere it has a flat nucleate epithelium and weaker musculature. Cement glands were not discerned.

Discussion:

The genus *Sabussowia* Böhmig, 1906 was erected to contain a single species, *S. dioica* (Claparède, 1863). Böhmig's definition is clear, if brief, and makes no mention of a major peculiarity of the type species, namely, that it is dioecious. The only other species of the genus hitherto known, *S. punctata* Brandtner, 1935, is monoecious, which is the usual condition within the Tricladida. A related form, *Cercyra teissieri* Steinmann, 1931, is also dioecious, the only other species within the Procerodidae that is so. Consequently, there is no difficulty in classifying the monoecious species from North America. Morphologically it fits perfectly within the genus *Sabussowia* as defined by both Böhmig and Brandtner, and Wilhelmi's (1909) enlargement of the definition of the genus to include dioeciousness is unfortunate.

The three species of the genus are similar in size; all are pigmented and with two eyes. They are small Maricola, ranging in length from 2 to 5 mm, although *S. dioica* may attain up to 7 mm. *S. wilhelmii* is most similar to *S. punctata*, from which it is most readily distinguished by its shorter common vas deferens, larger, though still thin-walled, bursa copulatrix, more numerous testes, and by the fact that only one branch of the intestine, the anterior main ramus, extends anteriorly to the eyes. In *S. punctata* the first pair of main lateral branches, just behind the ovaries, extend forwards anteriorly to the eyes (Brandtner 1935: 474, Fig. 2). Thus, a simple key to the known species of the genus *Sabussowia* may be provided:

1 Dioecious.....*S. dioica*
Monoecious.....2
2 Testes numerous; one preocellar intestinal duct.....*S. wilhelmii*
Testes few (<20); three preocellar ducts....*S. punctata*

The discovery of *Sabussowia* on the east coast of North America brings the number of free-living genera of Maricola that are common to both North America and Europe to four: *Sabussowia*, *Foviella*, *Uteriporus*, and *Procerodes*. The last three are represented in eastern North America by single species that are identical with forms found in Europe, and, thus, they show true amphi-Atlantic distributions.

Status of *Fovia warreni* (Girard, 1852)

The genus *Fovia* was established by Girard (1852) for a single species, *Vortex warreni* Girard, 1850, discovered on the shores of Boston Harbour. There has been confusion ever since as to the status of both the species and the genus. Subsequent to the original description the species has been recorded, under a variety of names, from many localities on the Atlantic coast of North America. Nonetheless, the species appears to have been found in the sexually mature state only once. Of over 100 specimens examined by Wilhelmi (1909) none, however, were fully mature, and a complete description was not possible. However, on the information available to him, he tentatively assigned the species to the genus *Procerodes* Girard, 1850b. This decision was accepted by Hyman (1944) without comment on Wilhelmi's uncertainty as to whether the species belonged in *Procerodes* or in *Stummeria* Böhmig, 1908.

Wilhelmi's main uncertainty concerned whether the oviducts opened separately or combined into the bursal stalk. Even if the former obtained, however, his species could not be assigned to *Stummeria* for it lacks the penial complexity and the ventrally located testes that are characteristic of the type species of this genus. In this case the species would revert to *Sabussowia* if, in addition, the vasa deferentia combined before entering the penis. Once more, however, this point is unclear (Wilhelmi 1909: 268, 338).

In general size, color, and bodyshape, and in the form and vertical disposition of the penis, the *P. warreni* of Wilhelmi is very similar to *S. wilhelmii*, but their common identity cannot be proven because crucial information for accurate generic assignment could not be obtained from Wilhelmi's material. Furthermore, there are some obvious differences. Wilhelmi (1909) makes no mention of the strong basal musculature of the penis, nor of the unusual width of the horizontally directed bursal stalk, which are so characteristic of *S. wilhelmii*. For these reasons I maintain, for the present, that Wilhelmi's species and mine are distinct. Moreover, I do not see how one can be sure that Wilhelmi's species is the same as that described by Girard (1850a).

The nomenclatural history of *Vortex warreni* is as utterly confusing as is that of two other species described in the nineteenth century: one from North America, *Procerodes wheatlandi* Girard; and one from Europe, *Fovia affinis* (Oersted). The former is now considered to be conspecific with *Procerodes littoralis* (Strom), and the latter is now known as *Foviella affinis* (Oersted) Bock, 1925. Bock correctly pointed out that *Fovia* could not be used for the *Planaria affinis* of Oersted as Girard had established *warreni* as the type. For detailed nomenclatural discussion the works of Wilhelmi (1909, 1911), Bock (1925, 1926) and Hyman (1944) may be consulted. What is important from the present point of view is that, subsequent to these discussions, two European species, *Uteriporus vulgaris* Bergendal, 1890 and *Foviella affinis*, involved in the many confusions, have been found to occur on the east coast of North America (Hyman 1944; Tomkiewicz and Ball 1972). Indeed, they are represented in the Naturhistoriska Riksmuseet, Stockholm, by specimens collected in 1911 by O. Hentschel from St John's, Newfoundland, the slide of *Uteriporus* originally having been labelled as *Procerodes warreni*?. With this in mind it now becomes impossible to ascertain if early North American workers were talking of species of *Procerodes*, *Uteriporus*, *Foviella*, *Sabussowia*, or *Fovia*, or even some indeterminate rhabdocoel! That confusion between these genera was rife is further evidenced by Gamble's (1893) identification of *Fovia affinis* with *U. vulgaris*, when in fact he had neither and was dealing with *S. dioica* (Wilhelmi 1908a; Hartog 1968). Most descriptions prior to Wilhelmi (1909) are vague and inconsistent in various ways. One may agree with Wilhelmi (1908b, 1909) that color variations are not in themselves adequate for the separation of the species, but his list of synonyms for *P. warreni* includes species that on size alone cannot reasonably be merged with one another.

In 1874 Verrill and Smith recognized both *F. warreni* and a new species, *Planaria grisea*. Girard (1893) later transferred the latter to his rhabdocoel genus *Neoplana* whereas, at the same time, Verrill (1893) considered *warreni* and *grisea* to be but varieties of the same species, *Fovia affinis*, a decision tentatively accepted by Böhmig (1906). Yet, in the original description, *Pl. grisea* is described as being 12 mm long, whereas Wilhelmi (1909) gives an upper limit of 4.5 mm for *P. warreni*. Moreover, *S. wilhelmii* and *P. warreni* are the smallest of the eastern North American Maricola, being greatly exceeded in size by both *P. littoralis* and *Foviella*

affinis. Yet, Girard's original description of *P. wheatlandi* (= *P. littoralis*) gives a maximum length of two lines, or 4.24 mm. *Planaria frequens*, also given by Wilhelmi (1909, 1911) as a synonym of *P. wheatlandi*, is described as not exceeding 4.24 mm by Leidy (1855) and 3.18 mm by Verrill and Smith (1874).

It seems most unlikely that either Girard or Verrill had any clear idea of the genera and species with which they were working. It appears that any pigmented marine planarian was assigned arbitrarily to *F. warreni*, *Fovia affinis* (= *Foviella*), *Pl. grisea*, or *P. wheatlandi*, almost on the basis of color alone, and without regard for critical characters or consistency of description. Moreover, these workers may have had any of these species to hand or even *U. vulgaris*. In view of this utter confusion, the objective solution of which may well be impossible, there seems no point in attempting to assign any of these early names either to the newly described species or to the one described by Wilhelmi (1909). Such an attempt would threaten nomenclatural stability within the genera *Procerodes*, *Uteriporus*, *Sabussowia*, and *Foviella*, without adding one iota to scientific knowledge.

Consequently I accept the decisions of Bock (1926) and Hyman (1944) concerning the status of *P. littoralis* and of Bock (1925) regarding *Foviella affinis*. *Fovia warreni* (Girard) and *P. warreni* of Wilhelmi are here proposed to be nomina dubia. I further propose that the generic name *Fovia* Girard, 1852 be considered a nomen oblitum in that it has remained unused as a senior synonym in the primary zoological literature for over fifty years, and I similarly recommend the suppression of the specific epithet in *F. warreni*. If Wilhelmi's *P. warreni* is later found to be identical with *S. wilhelmii* the latter name will take priority because Wilhelmi was using the trivial name in Girard's incomprehensible sense, and because the binomen has been used previously and, therefore, cannot be used for a new species (see synonymy in Wilhelmi 1909, 1911). In the event that Wilhelmi's species can be confirmed as being distinct from *S. wilhelmii*, then it may be described as new. Only in this way can the identity of the *P. warreni* in the works of Wilhelmi (1909), Hyman (1944), and Gosner (1971) be settled finally.

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Effects of a 12-hr and 25-day Exposure to Kraft Pulp Mill Effluent on the Blood and Tissues of Juvenile Coho Salmon (*Oncorhynchus kisutch*)

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McLeay, D. J. 1973. Effects of a 12-hr and 25-day exposure to kraft pulp mill effluent on the blood and tissues of juvenile coho salmon (*Oncorhynchus kisutch*). J. Fish. Res. Board Can. 30: 395–400.

Red blood cell counts and hematocrits of juvenile coho salmon were unaltered by 12-hr exposure to neutralized kraft pulp mill effluent, although hematocrits were decreased by exposure for 25 days. The number of circulating immature erythrocytes increased in effluent-exposed fish in both the 12-hr and 25-day exposures. The number of circulating small lymphocytes decreased markedly after 12-hr exposure. However, following the prolonged exposure, the number of small lymphocytes returned to normal, while the number of circulating neutrophils increased.

The level of plasma glucose increased in fish exposed to effluent for 12 hr, and decreased in fish exposed for 25 days. Liver glycogen was not altered significantly during either exposure period.

No pathological changes attributable to exposure to kraft pulp mill effluent were observed in the tissues examined, including the spleen, kidney, gill, interrenal gland, skin epithelium, and thyroid gland.

Results are discussed in terms of a stress response.

McLeay, D. J. 1973. Effects of a 12-hr and 25-day exposure to kraft pulp mill effluent on the blood and tissues of juvenile coho salmon (*Oncorhynchus kisutch*). J. Fish. Res. Board Can. 30: 395–400.

Le nombre de globules rouges sanguins et les hématocrites de jeunes saumons coho ne sont pas affectés par une exposition de 12 h à l'effluent neutralisé d'une usine de papier kraft. Par contre, il y a réduction d'hématocrites après exposition de 25 jours. Le nombre d'érythrocytes immatures en circulation augmente chez les poissons exposés à l'effluent durant 12 h et durant 25 jours. Il y a diminution notable des petits lymphocytes en circulation après exposition de 12 h. Cependant, après l'exposition plus longue, le nombre de petits lymphocytes redevient normal, alors que le nombre de neutrophiles en circulation augmente.

Le niveau de glucose plasmatique s'élève chez les poissons exposés à l'effluent durant 12 h, et diminue chez les poissons exposés durant 25 jours. Le glycogène hépatique n'est pas affecté de façon significative par l'une ou l'autre période d'exposition.

Nous n'avons observé aucun changement pathologique attribuable à l'exposition à l'effluent d'usine de papier kraft dans les tissus examinés, y compris la rate, les reins, les branchies, les corps interrénaux, l'épithélium de la peau et la thyroïde.

Les résultats sont analysés en termes de réponse à un stress.

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Neutralized kraft mill effluents are toxic to fish. Effluent discharge standards, derived by application factors based upon lethal concentrations as measured by conventional bioassays, do not necessarily ensure that fish populations in the receiving water will not be subjected to sublethal stresses which affect their survival.

Elucidation of the threshold concentrations at which physiological and biochemical changes occur in the blood or tissues of fish should provide a more sensitive, technically defensible method for assessing permissible discharge limits. Numerous physiological, biochemical, and histological changes in the blood and tissues of fish exposed to a variety of pollutants have been reported (Warner 1967; Katz et al. 1969; Sprague 1971). Fujiya (1961, 1964) described a variety of changes which occurred in the tissues of fish held in live boxes near a pulp mill outfall. Schaumberg et al. (1967) and Walden et al. (1970) used a buccal cannulation technique to monitor respiration changes in fish exposed to pulp mill effluent under laboratory conditions.

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Studies of acute effects of pollutants are useful in assessing the threshold of response to a particular toxicant. However, continued sublethal exposure may produce a variety of chronic symptoms not observed in short-term tests (Hassler et al. 1967), or may result in adaptation by the fish to the pollutant (McKim et al. 1970).

This study reports quantitative measurements of specified physiological and biochemical changes in the blood and tissues of fish exposed to sublethal concentrations of kraft pulp mill effluent under laboratory conditions.

Materials and Methods

Fish

Juvenile coho salmon (*Oncorhynchus kisutch*) were obtained from a hatchery and were held for several months in dechlorinated tap water at a temperature between 7 and 12 C. The fish were kept on a 12-hr photoperiod and were fed a commercial fish food (Oregon Moist pellets). Fish used in the 12-hr experiment were approximately 150 days old, measuring 6.6 ± 0.4 cm and 3.3 ± 0.6 g. Those used in the 25-day experiment were 225 days old, with a mean fork length of 8.7 ± 0.6 cm and mean weight of 7.3 ± 1.8 g when killed.

Pulp Mill Effluent

The pulp mill waste used in this study was neutralized unbleached white water collected from the main sewer of a coastal British Columbia kraft pulp mill. In order to retard degradation of this sample by microbial activity, its *p*H was adjusted from 9.0 to 4.0 with conc. H_2SO_4. The sample was stored in sealed containers at 2 C and *p*H 4 until used.

Experimental

Bioassays to establish the 96-hr median tolerance limit (TL50) value for the kraft pulp mill effluent were conducted as described by American Public Health Association (1971).

12-hr exposure — Ten fish were placed in each of six test compartments, $120\times10\times10$ cm, fitted with translucent covers. Effluent or dechlorinated tap water in 200-liter reservoirs was recirculated through these compartments at a rate of 2 liters/min. Dissolved oxygen was maintained above 9 mg/liter and temperature at 12 ± 1 C. During a 2-week acclimation period, fish were fed three times daily; water was renewed every 24 hr.

Fish in three compartments were exposed to pulp mill effluent for 12 hr. The effluent concentration was one-half of the sample's 96-hr median tolerance limit (TL50) value, determined by static bioassay. Fish in the remaining compartments served as controls.

25-day exposure — Acclimation procedures and test conditions for the 25-day exposure study were identical to those used in the 12-hr study. The effluent concentration used was about one-third of the sample's 96-hr TL50 value throughout the 25-day exposure period. Ten fish in each of three compartments were exposed to pulp mill effluent; fish in the remaining compartments served as controls.

Sampling — Wet weight and fork length were measured; the caudal peduncle was severed, and blood collected in a heparinized microhematocrit tube and a diluting pipette. A blood smear was prepared. The liver, kidney, head kidney, spleen, and lower jaw were taken for subsequent histological examination. Blood sampling was completed in less than 1 min; the entire autopsy procedure was performed in less than 5 min for each fish.

Blood counts — Total blood cell counts, red blood cell and white blood cell-thrombocyte counts were determined with a hemacytometer (Hesser 1960). White blood cell and thrombocyte counts were combined. Rees–Ecker blood diluting fluid (Klontz and Smith 1968) was used to distinguish cell types.

Blood smears were stained (Klontz and Smith 1968) and routine differential white blood cell counts determined. Subsequently, 3000 blood cells of all types were counted from each smear and classified as to type. The absolute differential cell count for each fish then was calculated by dividing the total number of blood cells/mm^3 by 3000, and multiplying this figure by the number of cells of each type as determined from the blood smear.

Hematocrit, plasma glucose — Blood samples were centrifuged at 12,500 rpm for 5 min, and hematocrits read directly. Plasma glucose was measured by the glucose oxidase microtechnique (Cawley et al. 1959).

Liver glycogen — Liver glycogen was determined according to the technique of Montgomery (1957).

Histometrics — Serial sections of tissues were cut at 5 μ using standard techniques. Sections were stained with Mallory Heidenhain's azan (Humason 1962) and counterstained with hematoxylin.

The diameters of 100 interrenal cell nuclei were measured for each fish. Mutually perpendicular diameters of ten nuclei, selected at random from each of 10 tissue sections, were measured directly at 1000× magnification. The minimum and maximum thickness of the lower jaw epithelium was measured from a single cross-section; the minimum thyroid follicle cell height of ten follicles was also measured directly at 1000× for each fish.

Data analysis — Mean and standard deviation values were calculated for each experimental group of 30 fish. Student's *t*-test was used to compare treatment values for each variable.

Results

Lengths, weights, and condition factors (cW/L^3) did not differ between control or effluent-exposed fish after either the 12-hr or 25-day exposure.

HEMATOLOGY

Total cell counts and RBC counts/mm³ blood did not differ in either 12-hr or 25-day exposures (Table 1). The number of immature RBCs/3000 cells was increased in exposed fish, for both periods. Hematocrits were unchanged after 12-hr exposure, but were decreased after 25-day exposure.

WBC-thrombocyte counts/mm³ blood in fish exposed for 12 hr were much lower than in the control group (Table 1). In the 25-day exposed fish the WBC-thrombocyte counts were not significantly different from controls.

The percentages of small lymphocytes, large lymphocytes, and neutrophils were unaffected by 12-hr exposure; however, after 25-day exposure, the percentage of small lymphocytes decreased, the percentage of neutrophils increased greatly and the percentage of large lymphocytes remained unchanged (Table 1).

The number of small lymphocytes/3000 cells and small lymphocytes/mm³ blood decreased markedly in fish exposed for 12 hr, but did not differ from control values after 25-day exposure (Table 1). After 12-hr exposure, counts of neutrophils/3000 cells and neutrophils/mm³ blood did not differ

TABLE 1. Effect of kraft pulp mill effluent[a] on blood cell counts of juvenile coho salmon[b] (*Oncorhynchus kisutch*).

	12-hr exposure		25-day exposure	
	Control	Effluent[c]	Control	Effluent[d]
	Absolute cell counts[e] (× 10^4 = *no./mm³ blood*)			
Total cell count	76.0±14.4[f]	71.0±13.4	104.1±18.4	98.2±20.5
RBC count	69.0±14.0	67.5±13.4	98.4±13.0	92.0±21.2
WBC-thrombocyte count	7.0±1.1	3.5±0.9***	5.7±1.1	6.2±1.9
	Differential WBC counts[g]			
Small lymphocytes (%)	96.1±2.8	94.6±3.8	95.6±2.5	90.2±5.1***
Large lymphocytes (%)	0.7±0.8	1.0±1.3	0.8±0.8	0.9±1.2
Neutrophils (%)	3.1±2.4	4.3±3.3	3.6±2.4	8.9±4.7***
	Total differential cell counts[g] (*no./3000 cells*)			
Mature RBCs	2644±137	2611±120	2848±62	2803±101
Immature RBCs	276±119	346±129*	87±43	131±79**
Small lymphocytes	39.4±19.5	11.0±9.9***	20.2±16.5	18.2±11.5
Large lymphocytes	0.3±0.6	0.2±0.5	0.2±0.5	0.4±0.8
Neutrophils	1.4±1.4	1.1±1.7	1.0±0.9	3.3±1.9***
Thrombocytes	38.9±26.4	30.6±24.2	43.9±29.2	43.9±31.4
	Absolute differential cell counts[h] (× 10^4 = *no./mm³ blood*)			
Mature RBCs	67.0±3.5	61.8±2.8	98.8±2.2	91.7±3.3
Immature RBCs	7.0±3.0	8.2±3.1	3.0±1.5	4.3±2.6*
Small lymphocytes	1.0±0.5	0.3±0.2***	0.7±0.6	0.6±0.4
Large lymphocytes	0.008±0.02	0.005±0.01	0.007±0.02	0.01±0.03
Neutrophils	0.04±0.04	0.03±0.04	0.03±0.03	0.11±0.06***
Thrombocytes	1.0±0.7	0.7±0.6	1.5±1.0	1.4±1.0

[a]Neutralized kraft mill unbleached white water, 30% v/v.
[b]Thirty fish/sample, acclimated to test compartments for 2 weeks prior to effluent addition.
[c]Toxicity one-half of 96-hr TL50.
[d]Toxicity one-third of 96-hr TL50.
[e]Determined at autopsy by hemacytometer.
[f]Mean ± standard deviation.
[g]Determined from blood smears.
[h]Determined by calculation.
P values based on student's *t*-test represent significance of difference between control and effluent-exposed samples. *$P<0.05$; **$P<0.01$; ***$P<0.001$.

between control and effluent-exposed fish, but both counts were elevated in fish exposed to effluent for 25 days.

The number of large lymphocytes/3000 cells, large lymphocytes/mm^3 blood, thrombocytes/3000 cells, or thrombocytes/mm^3 was not altered by either exposure (Table 1).

Plasma Glucose and Liver Glycogen

Plasma glucose levels were increased in fish exposed to effluent for 12 hr (Table 2). After 25-day exposure, glucose levels were decreased.

Mean values for liver glycogen content were not altered by either exposure (Table 2).

Histopathology

No changes were observed in the histology of the spleen, kidney, head kidney, gill tissue, interrenal tissue, skin epithelium, or thyroid gland of fish exposed to effluent for 12 hr or 25 days. In addition, measurements of nuclear diameters of interrenal cells, thickness of skin epithelium, and thyroid follicle cell height were not altered by either exposure (Table 2).

Discussion

Decreases in hematocrit values of fish receiving prolonged exposure to pulp mill effluent have been noted previously (Warner 1967). A significant decrease in the red blood cell count and hematocrit was also found previously in yearling coho salmon exposed to pulp mill effluent for 14 days (D. J. McLeay unpublished data). These effects could be due to an inhibition of erythrocyte production, an increase in the rate of erythrocyte destruction, or hemodilution.

The increase in the number of circulating immature erythrocytes could reflect a stimulation of erythropoiesis by elevated demands for O_2 or CO_2 transport in effluent-exposed fish as a result of increased physical or metabolic activity or by destruction of gill membranes causing faulty gaseous exchange. Erythropoiesis could also be stimulated by an increased fragility or rate of destruction of circulating erythrocytes.

The decrease in the leukocyte–thrombocyte count during 12-hr exposure to pulp mill waste is mainly due to a decrease in the number of circulating small lymphocytes; counts of other cell types including thrombocytes were not decreased significantly. This decrease may be due to a stress-mediated increase in secretion of corticosteroids by the interrenal tissue. Both stress and corticosteroid administration have been shown to cause lymphopenia in mammals (Dougherty and White 1943, 1944) and salmonid fishes (Weinreb 1958; McLeay 1970).

A variety of stressors stimulate the adrenal tissue of teleost fishes, resulting in increased levels of circulating glucocorticoids (McKim 1966; Fagerlund

Table 2. Effect of kraft pulp mill effluent[a] on biochemical and histometric parameters of juvenile coho salmon[b] (*Oncorhynchus kisutch*).

	No. Fish	12-hr exposure		25-day exposure	
		Control	Effluent[c]	Control	Effluent[d]
Hematocrit (%)	30	21.1±3.1[e]	21.3±1.9	26.2±2.1	24.5±2.7**
Plasma glucose (mg%)	30	54.2±8.2	82.8±25.4***	60.8±10.2	55.5±9.5*
Liver glycogen (*mg/g liver wt*)	30	70.7±16.9	71.3±24.2	37.5±9.4	33.4±16.5
Nuclear diameter of interrenal cells (μ)	30	6.4±0.3	6.4±0.3	6.5±0.2	6.4±0.2
Thickness of skin epithelium (μ)	15	90.5±22.5	79.5±18.0	93.5±34.0	91.0±27.0
Thyroid follicle cell height (μ)	15	3.1±0.9	3.2±0.9	2.5±0.8	2.4±0.8

[a]Neutralized kraft mill unbleached white water, 30% v/v.
[b]Acclimated to test compartments for 2 weeks prior to effluent addition.
[c]Toxicity one-half of 96-hr TL50.
[d]Toxicity one-third of 96-hr TL50.
[e]Mean ± standard deviation.
P values based on student's *t*-test represent significance of difference between control and effluent-exposed samples.
*$P<0.05$; **$P<0.01$; ***$P<0.001$.

1967; Wedemeyer 1969) and catecholamines (Nakano and Tomlinson 1967). Both of these groups of hormones produce hyperglycemia (Falkmer 1961; Young and Chavin 1965). It is speculated that the short-term (12-hr) exposure of fish to pulp mill effluent caused a stress induced, hormonally mediated elevation of plasma glucose levels.

Catecholamines can produce a rapid depletion in liver glycogen of fish (Stimpson 1965; Nakano and Tomlinson 1967). Since liver glycogen stores in juvenile coho salmon were unaffected by a 12-hr exposure to pulp mill wastes, the observed hyperglycemic response may have resulted from gluconeogenesis as a consequence of increased glucocorticoid output, rather than being related to glycogenolysis subsequent to increased adrenaline secretion.

Numerous researchers have described pathological changes in tissues of fish exposed to a variety of pollutants at sublethal levels. Fujiya (1961, 1964) observed necrosis of gill filaments, degeneration of kidney tubules, internal hemorrhages in spleens, necrosis and desquamation of epithelium, and other degenerative changes in fish exposed to pulp mill effluent for 12 or 24 hr. Unlike these observations, no histopathological changes whatsoever were observed following exposure of fish to neutralized kraft mill effluent for 12 hr or 25 days, although highly significant differences in blood cell counts and blood sugar levels were found in the same fish. Since hyperglycemia and lymphopenia are characteristic responses to increased secretory activity of the adrenal glands in mammals (Selye 1950; Oguri and Nace 1966), it was anticipated that the interrenal tissue of juvenile coho salmon exposed to pulp mill wastes for 12 hr would appear more active than that of control fish. However, subjective criteria of activity and measurements of interrenal cell nuclei failed to indicate increased adrenal gland activity. Thus, this short-term exposure may not have stimulated the interrenal tissue; on the other hand, the 12-hr exposure may not have allowed sufficient time for a measurable increase in nuclear diameters of interrenal cells, although increased corticosteroid output by these cells during this period resulted in the measured hyperglycemia and lymphopenia. Elevation of plasma cortisol levels with no accompanying changes in the histology of the interrenal gland was noted in juvenile coho salmon during an initial 4-day exposure to cold temperatures; a 14-day exposure was required before the nuclear diameters of interrenal cells showed a corresponding increase (Allan 1971).

An initial elevation of plasma corticosteroid levels in fish exposed to pulp mill effluent is probable, although not yet established. Transient elevations in plasma "cortisol" (Hill and Fromm 1968), and increases in stress-hormone metabolites in urine (McKim 1966) have been measured in fish after acute exposure to sublethal levels of other pollutants. These results, together with the present findings, support the hypothesis that initial exposure of fish to pulp mill effluent and other pollutants can evoke a characteristic stress response. The effects and consequences of prolonged exposure to pollutants, particularly with regard to pulp mill effluent, remain ill-defined.

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Inheritance of Thermal Resistance in Hybrids of *Salvelinus fontinalis* and *S. namaycush*[1,2]

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IHSSEN, P. 1973. Inheritance of thermal resistance in hybrids of *Salvelinus fontinalis* and *S. namaycush*. J. Fish. Res. Board Can. 30: 401–408.

The two reciprocal F_1 hybrids of brook trout (*Salvelinus fontinalis*) and lake trout (*S. namaycush*) had similar times to death on exposure to several lethal high temperatures for a series of acclimation temperatures. These hybrids resembled the brook trout, the more resistant parent, more than the lake trout. The F_2 hybrids were intermediate in resistance to the parent species, and the backcrosses intermediate between the F_2 hybrids and the respective parents. After acclimation to 5 and 10 C, F_2's and backcrosses arising from the F_1 hybrid of brook trout maternal origin were consistently higher in resistance than the F_2 and backcrosses arising from the F_1 hybrid of lake trout maternal origin. After acclimation to 20 C and above, differences associated with the maternal origin of the F_1 hybrid were not found.

The number of effective factors segregating was estimated using the techniques of variance component analysis. A genetic model with two codominant factors was found to fit the data for 5 and 10 C acclimation, and one with five factors and dominance of the brook trout factors was found to fit the data for the higher acclimation temperatures.

IHSSEN, P. 1973. Inheritance of thermal resistance in hybrids of *Salvelinus fontinalis* and *S. namaycush*. J. Fish. Res. Board Can. 30: 401–408.

Les deux hybrides réciproques de F_1 ont des durées semblables de résistance à la mort lorsqu'exposés à plusieurs températures létales élevées, pour une série de températures d'acclimatation. Ces hybrides ressemblent plus à l'omble de fontaine, le parent le plus résistant, qu'au touladi. Les hybrides de F_2 ont une résistance intermédiaire à celle des espèces parents, et les descendants de croisements de retour une résistance intermédiaire à celle des hybrides de F_2 et de leurs parents respectifs. Après acclimatation à 5 et 10 C, les hybrides de F_2 et les descendants de croisements de retour issus de l'hybride de F_1 de l'omble de fontaine origine maternelle ont une résistance uniformément plus forte que les hybrides de F_2 et les descendants de croisements de retour issus de l'hybride de F_1 du touladi origine maternelle. Après acclimatation à 20 C et plus, les différences associées à l'origine maternelle de l'hybride de F_1 ne peuvent être détectées.

Nous avons estimé le nombre de facteurs effectifs de ségrégation, à l'aide des techniques de l'analyse des composants de la variance. Un modèle génétique à deux facteurs codominants s'accorde avec les données d'acclimatation à 5 et 10 C. Un autre à cinq facteurs, avec dominance des facteurs de l'omble de fontaine, s'accorde avec les données d'acclimatation aux températures plus élevées.

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THE purpose of this study was to examine the inheritance of thermal resistance by applying quantitative genetic techniques to the variation in resistance found among the hybrids of the brook trout (*Salvelinus fontinalis*) and the lake trout (*S. namaycush*). The times to death on exposure to a series of lethal high temperatures for several acclimation temperatures are substantially different for these two species (Fry and Gibson 1953). Intraspecific differences are comparatively small, however, as was found by McCauley (McCauley 1958) for the brook trout and confirmed in the present study for both species. This conservative nature of thermal resistance has been demonstrated for various poikilotherms by several other workers (Hart 1952; Ushakov 1964; Bidgood and Berst 1969).

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The genetic nature of such interspecific differences is open to analysis using standard quantitative genetic techniques if the interspecific hybrid is fertile and shows normal Mendelian segregation of parental alleles. The F_1 hybrid (splake) is comparable in fertility to the parent species (Buss and Wright 1956; Ayles personal communication), and normal Mendelian segregation of parental alleles has been reported for LDH isozyme phenotypes (Morrison and Wright 1966) and visual pigment phenotypes (McFarland and Munz 1965).

Materials and General Methods

Two brook trout populations were used, one from Hill's Lake Fish Hatchery, Ontario Department of Lands and Forests, and the other a wild population from Dixon Lake, Algonquin Park, Ontario. Individuals from these populations are denoted by B^H and B^D, respectively. Lake trout were obtained from the wild populations of Lake Opeongo (L^O) and Lake Louisa (L^L), Algonquin Park, Ont. The two reciprocal F_1 splake stocks used were derived from the Hill's Lake and Lake Louisa populations and are denoted by BL and LB, the first initial referring to the maternal parent.

Crosses between individuals chosen randomly from the above stocks were made by artificial fertilization in the fall of 1966, 1967, and 1968 (Table 1). In all three years the same temperature (9 ± 1 C) was maintained during fertilization, incubation, and rearing. Other environmental conditions such as feed, photoperiod, and water quality were also the same for all crosses and each year-class. The fry were reared to approximately 4 cm before they were used in the experiments.

The test temperatures were chosen such that the highest produced 100% mortality in a few hours and the lowest at least 50% mortality within 10 days. Deaths were recorded at equal logarithmic time intervals according to the procedure of Scott (1964) and Tyler (1966). The criterion for death was the complete cessation of opercular movement. Each test continued until the last fish had died or for 20,000 min, after which time fish still surviving were considered to have adjusted to the test temperature.

Tests at each temperature were replicated at least twice, usually three times. The number of individual fish per cross for a given replicate experiment ranged from 10 to 40. All tests within each acclimation temperature were performed randomly and over as short a time period as possible (approximately 10 weeks). This minimized possible confounding effects of the changing natural light cycle.

The rate of acclimation used for these experiments was 1 C/day until within 2 degrees of the final acclimation temperature, and then 0.5 C/day. The fish were held at the final temperature for a minimum of 2 weeks before tests were started. The acclimation temperatures used were 5, 10, 20, and 23 C. During acclimation and testing, the fish were exposed to the natural light cycle at 44° N latitude.

TABLE 1. List of crosses tested. In each cross the female parent is written first. The lake trout parents are denoted[a] by L^O and L^L and brook trout parents by B^H and B^D. The F_1 parents are denoted by LS and SL with the female parent written first.

Female parent	Male parent: B	L	BL	LB
B	$B^H \times B^H(67^b)(2^c)$	$B^H \times L^L(66)(1)$	$B^H \times BL(66)(1)$	$B^H \times LB(66)(1)$
	$B^H \times B^H(68)$ (4)	$B^H \times L^L(67)(2)$	$B^H \times BL(67)(1)$	$B^H \times LB(67)(1)$
	$B^D \times B^D(68)$ (2)	$B^H \times L^L(68)(2)$	$B^H \times BL(67)(1)$	$B^H \times LB(68)(3)$
	$B^D \times B^H(68)$ (1)	$B^D \times L^O(68)(2)$	$B^H \times BL(68)(3)$	$B^D \times LB(68)(1)$
		$B^H \times L^O(68)(2)$	$B^D \times BL(68)(1)$	
L	$L^L \times B^H(66)$ (1)	$L^L \times L^L(67)(2)$	$L^L \times BL(67)(1)$	$L^L \times LB(67)(1)$
	$L^L \times B^H(67)$ (2)	$L^L \times L^L(68)(2)$	$L^L \times BL(68)(1)$	$L^O \times LB(68)(1)$
	$L^L \times B^H(68)$ (1)	$L^O \times L^O(68)(3)$	$L^O \times BL(68)(1)$	
	$L^L \times B^D(68)$ (1)			
	$L^O \times B^H(68)$ (3)			
	$L^O \times B^D(68)$ (1)			
BL	$BL \times B^H(66)$ (1)	$BL \times L^L(66)(1)$	$BL \times BL(66)(1)$	$BL \times LB(66)(1)$
	$BL \times B^H(67)$ (1)	$BL \times L^L(67)(1)$	$BL \times BL(67)(1)$	$BL \times LB(67)(1)$
	$BL \times B^H(68)$ (2)		$BL \times BL(68)(1)$	$BL \times LB(68)(1)$
LB	$LB \times B^H(67)$ (2)		$LB \times BL(67)(2)$	$LB \times LB(67)(1)$
				$LB \times LB(68)(6)$

[a]The superscripts refer to origins of the stocks: O, Lake Opeongo, Ont; L, Lake Louisa, Ont.; H, Hill's Lake, Ont.; D, Dixon Lake, Ont.
[b]Year of spawning.
[c]No. of replicates.

Times to Death

The resistance of each cross was characterized independent of test temperature for each acclimation temperature by the mean log time to death for all test temperatures. This was possible because analyses of variance showed no significant interaction between the log responses for all crosses and test temperatures within each acclimation temperature[4]. Therefore, all crosses (including parentals) were parallel in log response over test temperatures within acclimation temperatures. The test temperature ranges over which these means were calculated are 23.5–25.5, 25.5–26.7, and 27.5–28.7 C for acclimation to 5, 10, and 20 C, respectively. Although some of the crosses were also tested at 23 C acclimation, these results are omitted from this discussion because they were similar to those at 20 C acclimation. Since the 1969 test temperatures differed slightly from those for the other two years, the means for 1969 were interpolated to the mean test temperatures of the 1967 and 1968 experiments.

The data collected in different years and for replicates of the same cross were pooled (Table 2). Comparison of resistances for crosses produced and tested in different years generally showed no year to year differences.[5] Also, no differences in resistance were found among the parental as well as hybrid crosses derived from the different geographic populations. Hart (1952), McCauley (1958), and Bidgood and Berst (1969) found the same intraspecific uniformity in resistance to high temperatures for populations of the same species of different geographic origin.

Times to Death

The resistances of the backcrosses to the brook trout and lake trout, denoted by $B_1(B)$ and $B_1(L)$, respectively, and of the F_1 and F_2 hybrids relative to the parent species are indicated by the inequalities

$$B\times B > B_1(B) > F_1 > F_2 > B_1(L) > L\times L$$

Highly significant differences among the hybrids within groups $B_1(B)$, F_2, and $B_1(L)$ were found at 5 and 10 C acclimation. For example, after acclima-

[4]Two cases of nonparallelism omitted from further analyses in this paper are as follows. The F_1 hybrid L×B at the highest test temperature used for 5 C acclimation (25.5 C) was lower in resistance than expected for a parallel response. The most resistant crosses at the lowest test temperatures sometimes had higher rates of increase in log resistance with decreasing test temperatures than the less resistant crosses over the same test temperatures. This latter effect is thought to be caused by additional acclimation during experimental exposure since the rates of acclimation calculated from these deviations from parallelism agree closely with those in the literature (Brett 1946; Cocking 1959). For details of calculation see Ihssen (1971).

[5]Crosses tested in the summer were sometimes slightly more resistant than replicates of the same cross tested in the spring of another year (Ihssen 1971).

TABLE 2. Mean log times to death in minutes with their standard errors.

Hybrid type	Cross	Acclimation temp (C) 5	10	20
	B×B	2.113±0.015	2.053±0.012	2.063±0.009
$B_1(B)$	B×BL	1.939±0.026[a]	1.938±0.019	1.971±0.012
	BL×B	1.898±0.026	1.914±0.022	1.985±0.015
	B×LB	1.754±0.031	1.816±0.021	1.969±0.015
	LB×B	1.601±0.022	1.717±0.025	1.983±0.017
F_1	B×L	1.755±0.022	1.895±0.016	1.881±0.011
	L×B	1.744±0.027	1.908±0.017	1.867±0.012
F_2	BL×BL	1.617±0.029	1.730±0.031	1.802±0.017
	BL×LB	1.670±0.033	1.732±0.024	1.803±0.017
	LB×BL	1.371±0.030	1.563±0.025	1.791±0.017
	LB×LB	1.327±0.043	1.598±0.018	1.798±0.016
$B_1(L)$	BL×L	1.392±0.031	1.589±0.035	1.689±0.025
	L×BL	1.295±0.031	1.621±0.027	1.669±0.025
	L×LB	1.169±0.031	1.599±0.031	1.659±0.025
	L×L	1.140±0.025	1.436±0.018	1.456±0.025

[a]Crosses included in each bracket are not significantly different in resistance.

tion to 5 C for the test temperature 23.5 C, the mean times to death in minutes for the B_1(B) hybrids B×BL,[6] BL×B, B×LB, and LB×B were 287, 230, 161, and 126, respectively. The relation among the hybrids within the three groups at 5 C acclimation is represented by the inequalities below:

B_1(B): B×BL≃BL×B>B×LB > LB×B
B_1(L): BL×L > L×BL > L×LB
F_2: BL×BL ≃ BL×LB > LB×BL ≃ LB×LB

The differences diminish with increasing acclimation temperature until at 20 C acclimation significant differences disappear.

The above inequalities indicate that the differences are associated with the origins of both the maternal and paternal grandparents. The maternal grandparent being a lake trout (e.g. LB×B), rather than a brook trout (e.g. BL×B), lowers the resistance of the hybrids in each group. The maternal origin of the male parent has a similar effect but only within groups B_1(B) and B_1(L) and, also, this male effect is less persistent with increasing acclimation temperatures than is the female effect.

The Genetic Model

For the construction of the genetic model it was assumed that the parent species could be considered to be true breeding lines with respect to thermal resistance. This assumption is a good approximation because of the conservative nature of thermal resistance, and also because it was found in this study that the differences in thermal resistance among replicate crosses were small compared to the difference between the parent species. Differences in resistance among replicate crosses (whatever their origin) were of the order of 0.04 log min or approximately 10% difference in time to death, whereas the difference between the parent species varied from 0.6 log min up to 1.0 log min or a factor from 4 to 10 in time to death.

Transformation of Data

The log transformed data satisfy the inertia necessary for the variance component analysis that follows. The variances of log times to death of the parental as well as the F_1 hybrids are approximately equal within years and acclimation temperatures[7] (Table 3). Also, the parental and F_1 log times to death were found to be normally distributed. Because of the assumption of intraspecific genetic homogeneity, these variations in resistance are regarded as nongenetic effects and considered to estimate the environmental variation.

For 1969 the parental and F_1 variances were slightly smaller than the 1968 variances (Table 3). This difference is attributed to a refinement in the testing technique introduced in 1969 (Ihssen 1971).

Another criterion satisfied by the log transformed data is that the effect of a particular gene substitution is independent of the rest of the genotype. This is called the additivity condition (Mather 1949) and was tested by comparing the phenotypic means of the nonsegregating crosses (B×B, L×L, F_1) with the F_2 and backcross hybrids. It was found that the additivity condition was satisfied with two

[6]Notation: an "×" links the parents of each cross, the female parent being written first.

[7]The small but significant difference in the variance at 20 C acclimation is dealt with in the discussion of the genetic model.

Table 3. Estimates of the phenotypic variances of log time to death in minutes. These variances were calculated from the variances of replicate experiments that were found to be homogeneous for each group of hybrids and the parent species.

	5 C acclimation	10 C acclimation		20 C acclimation	
Lot	1968	1968	1969	1968	1969
B×B	0.0178(443[a])	0.0093(493)	0.0074(885)	0.0038(187)	0.0025(546)
L×L	0.0185(292)	0.0108(255)	0.0078(421)	0.0058(82)	not tested
F_1	0.0164(538)	0.0097(584)	0.0074(1336)	0.0048(424)	0.0039(763)
B_1(B)	0.0423(936)	0.0201(551)	0.0170(1241)	0.0056(662)	0.0051(863)
B_1(L)	0.0468(688)	0.0215(605)	0.0196(321)	0.0145(261)	not tested
F_2	0.0740(987)	0.0352(524)	0.0335(712)	0.0145(477)	0.0137(222)

[a]The number of degrees of freedom associated with the variance.

exceptions. At 5 and 10 C acclimation those crosses that exhibited the lake trout maternal or paternal grandparent effect did not satisfy the additivity condition. However, with increasing acclimation temperatures and diminishing grandparental effect all these crosses also satisfied the additivity condition. Secondly, at 5 and 10 C acclimation the F_1 crosses do not satisfy the additivity conditions. Again, this effect disappears at 20 C acclimation. This deviation of the F_1 hybrid was attributed to heterotic effects.

Variance Component Analysis

The phenotypic variation of a quantitative character measured on a scale that satisfied the above criteria can be divided into four components (Serra 1966). These are: E, the component due to nonheritable variation; A, the additive component due to the difference between parental homozygotes for each locus; D, the dominance component due to the difference between heterozygotes and the average of the two homozygotes for each locus; and AD, the interaction between the additive and dominance effects.

The phenotypic variance of each hybrid can be expressed as a linear function of these components (Kempthorne 1957):

$$\begin{aligned} V[B_1(B)] &= \tfrac{1}{2}A+D-\tfrac{1}{2}AD+E \\ V[B_1(L)] &= \tfrac{1}{2}A+D+\tfrac{1}{2}AD+E \\ V[F_2] &= A+D+E \end{aligned}$$

$V[B_1(B)]$, $V[(B_1(L)]$, and $V[F_2]$, the phenotypic variances of the groups of hybrids in brackets, were estimated by the variances in log times to death of the backcrosses to the brook trout, the backcrosses to the lake trout, and the F_2 hybrids. E was estimated as the mean phenotypic variance of the parent species and their F_1 hybrids. Estimates of A, D, and AD were calculated from the above equations (Table 4). The estimates of A, D, and AD for 1968 and 1969 were not significantly different.

In order to put forth a specific genetic model, a minimal estimate of the number of factors segregating was calculated by the method according to Mather (1949) and given by Serra (1966). These "factors" could be single genes or groups of genes segregating as a unit.

The method employs the relation $n = \dfrac{P}{8A}$ where n is the minimum number of factors segregating, P the phenotypic difference of the two extreme genotypes (here the difference in log mean time to death of the parent species), and A the additive genetic variance. Substituting the estimates of P and A in the above equation the values of n given below were obtained:

Acclimation temp (C)	5	10	20
$\hat{n}$	2.1	1.7	5.1

Hence, a model with a minimum of two factors was sought at 5 and 10 C, and one with five at 20 C. Since at 5 and 10 C acclimation the components D and AD were approximately 0, a model with two factors of equal and additive effects with no dominance was tested against the data for these acclimation temperatures. At 20 C acclimation, where D and AD contributed a significant percentage to the total genetic variance (53%), a model with five factors of equal and additive effects with dominance of the brook trout factors was tested.

TABLE 4. Estimates of variance components from log times to death for acclimation temperatures (5–20 C) and the two years.

Variance component[a]	5 C	10 C		20 C	
	1968	1968	1969	1968	1969
A	0.0589	0.0288	0.0304	0.0089	–
D	0[b]	0	0	0.0010	–
AD	0	0	0	0.0089	–
E	0.0174	0.0980	0.0075	0.0046	0.0033

[a]A, D, and AD are the genetic components of variance additive, dominance, and additive × dominance interaction respectively; E is the nongenetic component of variance.

[b]Small positive and negative estimates for D and AD were set at zero.

Testing the Model

On the assumption of equality and additivity of the factors each genotype with a distinct phenotypic effect can be represented by the aggregate (i, j, k) where i, j, and k are integers representing, respectively, the number of loci homozygous for brook trout, heterozygous, and homozygous for lake trout, such that $i+j+k = n$, the number of factors. Then e.g. the brook trout, lake trout, and F_1 genotypes are represented by $(n,0,0)$, $(0,0,n)$ and $(0,n,0)$, respectively. Setting the mean phenotypic effect of the lake trout genotype arbitrarily at the origin and letting the additive and dominance effects of a single factor substitution from lake trout to brook trout be represented by "a" and "d," respectively, then the mean phenotypic effect of any genotype (i, j, k)

$$x_{i,j,k} = i(2a)+j(a+d).$$

The value of a is estimated by $\hat{P}/2n$ where $\hat{P}$ is the difference in log mean time to death of the parent species. At 5 and 10 C acclimation $d = 0$ because of the assumption of no dominance, and at 20 C acclimation is estimated by $\hat{P}(F_1)/n-\hat{a}$ where $\hat{P}(F_1)$ is the difference in log time to death between the F_1 hybrid and the lake trout parent.

Let $N(x,i,j,k)$ represent the phenotypic distribution of individuals with genotype (i,j,k) and let $p_{i,j,k}$ be the frequency of this genotype. $N(x,i,j,k)$ is assumed to be normally distributed since the phenotypic distributions of the parental and F_1 genotypes were found to be normally distributed. Letting the variances of these distributions be represented by $\sigma^2_{i,j,k}$, then

$$N(x,i,j,k) = \frac{1}{\sqrt{2\pi\,\sigma^2_{i,i,k}}} \exp\left(\frac{1}{2\sigma^2_{i,j,k}}[x-x_{i,j,k}]^2\right),$$

the normal distribution with mean $x_{i,j,k}$ and variance $\sigma^2_{i,j,k}$.

The $\sigma^2_{i,j,k}$'s were estimated from the observed phenotypic variances of the parentals and F_1 hybrids by linear interpolation as indicated in the equation

TABLE 5. Comparison of the distributions of the observed numbers of deaths and those predicted by the genetic model, along with the observed and predicted means and variances of the distributions.

Hybrid type	Hybrid	Year	χ^2	Mean		Variance	
				Observed	Predicted	Observed	Predicted
			Acclimation temp 5 C				
$B_1(B)$	B×BL, BL×B	1968	25.0 (21[a])	1.888	1.869	0.0455	0.0426
	B×LB	1968	20.7 (16)	1.754	–	0.0399	0.0426
	LB×B	1968	26.6[b](16)	1.601	–	0.0369	0.0426
$B_1(L)$	BL×L	1968	11.0 (17)	1.392	1.382	0.0446	0.0426
	L×BL	1968	12.8 (14)	1.295	–	0.0526	0.0426
	L×LB	1968	16.4 (14)	1.169	–	0.0413	0.0426
F_2	BL×BL, BL×LB	1968	34.0 (26)	1.531	1.626	0.0749	0.0771
	LB×BL, LB×LB	1968	25.4 (21)	1.349	–	0.0725	0.0771
			Acclimation temp 10 C				
$B_1(B)$	B×BL, BL×B	1968	4.8 (12)	1.892	1.877	0.0215	0.0213
	B×BL, BL×B	1969	21.1 (14)	1.959	1.877	0.0161	0.0189
	B×LB	1968	19.1[b](9)	1.788	–	0.0169	0.0213
	B×LB	1969	14.1 (11)	1.851	–	0.0189	0.0189
	LB×B	1968	10.6 (9)	1.717	–	0.0208	0.0213
$B_1(L)$	BL×L, L×BL, L×LB	1968	19.5 (14)	1.588	1.575	0.0215	0.0213
	L×BL, L×LB	1969	9.2 (12)	1.604	1.575	0.0196	0.0189
F_2	BL×BL, BL×LB	1968	11.7 (16)	1.720	1.726	0.0340	0.0327
	BL×BL, BL×LB	1969	14.8 (14)	1.751	1.726	0.0310	0.0303
	LB×BL, LB×LB	1968	17.2 (12)	1.580	–	0.0388	0.0327
	LB×LB	1969	24.1 (15)	1.610	–	0.0341	0.0303
			Acclimation temp 20 C				
$B_1(B)$		1968	11.7 (7)	1.979	1.965	0.0049	0.0060
$B_1(B)$		1969	20.2 (14)	1.953	1.965	0.0051	0.0051
$B_1(L)$		1968	15.1 (10)	1.664	1.667	0.0145	0.0145
F_2		1968	19.8[b](11)	1.802	1.816	0.0147	0.0155
F_2		1969	16.5 (19)	1.786	1.816	0.0137	0.0141

[a]Number of degrees of freedom of the χ^2.
[b]Significant at the 5% level.

$$\hat{\sigma}^2_{i,j,k} = \frac{i\,\hat{\sigma}^2_{B\times B} + j\,\hat{\sigma}^2_{F_1} + k\,\hat{\sigma}^2_{L\times L}}{i+j+k}$$

where $\hat{\sigma}^2_{B\times B}$, $\hat{\sigma}^2_{F_1}$, and $\hat{\sigma}^2_{L\times L}$ are the observed phenotypic variances of the brook trout, F_1 hybrid, and lake trout, respectively.

The $p_{i,j,k}$'s are given by $(\frac{1}{2})^n\binom{n}{j}$ for the groups of hybrids $B_1(B)$ and $B_1(L)$, and $(\frac{1}{4})^{i+k}\,(\frac{1}{2})^j\,\frac{n!}{i!j!k!}$ for the F_2 hybrids.

The model for the distribution of individual deaths in the segregating populations is given by the relation

$$D(x,\text{cross}) = \sum_{i,j,k} p_{i,j,k}\ N(x,i,j,k)$$

where "cross" determines the particular set of aggregates (i,j,k) over the integers of which the sum is taken.[8] Substituting the estimates of $x_{i,j,k}$ and $\sigma^2_{i,j,k}$ (note these estimates are obtained from the data of the parentals and the F_1 hybrids only), the predicted distributions of deaths for the hybrids can be calculated. For those hybrids that satisfy the additivity condition the model also predicts the mean times to death.

The expected number of deaths in the log time interval t_1–t_2 for a population of size N is given by

$$N \int_{t_1}^{t_2} \hat{D}(t,\text{cross})\,dt$$

where $\hat{D}$ indicates that the model with the estimated $x_{i,j,k}$'s and $p_{i,j,k}$'s is used. Since the interval lengths used to obtain the observed distributions of deaths were small (either 0.05 or 0.025 log min), it was found sufficient to estimate the integral by $D(\bar{t}, \text{cross}) \times (t_2-t_1)$ where $\bar{t}$ is the middle of the log interval.

The expected distributions of deaths as calculated from the model and the observed distributions of deaths were compared using the χ^2 goodness of fit test (Snedecor 1957). The observed distributions of deaths were obtained by combining the distributions from all replicate experiments for a particular cross by translating the mean of each replicate to the mean of that cross and summing the deaths in each log interval over replicates. Crosses whose mean log resistance times were not significantly different were also combined.

[8]e.g. For the hybrids $B_1(L)$ and a two factor model the aggregates are (0,2,0), (0,1,1), (0,0,2) corresponding to the genotypes BL BL, BL LL, LL LL, respectively.

The model predicts the distributions of deaths as well as the mean log times to death of those crosses that satisfy the additivity condition very accurately (only one in 14 observed distributions is significantly different from the predicted at the 5% level) (Table 5). The shapes of the distributions of those crosses that do not satisfy the additivity condition, i.e. the crosses derived from LB F_1 parents that show the lake trout grandmaternal effect at 5 and 10 C acclimation, are also fairly accurately predicted by the model (only two of 10 observed distributions differ significantly from the predicted at the 5% level) (Table 5). Hence, it appears that the LB F_1 parent simply shifts the means of the distribution of deaths of these hybrids towards the mean of the lake trout parent without affecting the variation among individuals of these hybrids.

Two hypotheses were put forward to account for this effect: firstly, one involving changes in the genotype frequencies i.e. one affecting the $p_{i,j,k}$'s in the model; and, secondly, one involving the phenotypic expression of the different genotypes i.e. one affecting the $x_{i,j,k}$'s in the model.

The first hypothesis implies that the hybrids derived from the F_1 of lake trout maternal origin (i.e. LB) have more lake trout genetic material than expected from random assortment, not only for the loci involved in high temperature resistance but for all loci. Hence, it is expected that at the higher acclimation temperatures these differences in resistance persist whether the loci involved in resistance at the higher acclimation temperatures are the same or not. However, as was discussed earlier, no such differences were found at 20 and 23 C acclimation. Hence, more complicated mechanisms must be invoked for this hypothesis, and for this reason the following second hypothesis is preferred.

The second hypothesis implies that cytoplasmic and or nuclear modifiers associated with the lake trout female, and, perhaps, interactions between these, affect the phenotypic expression of genotypes derived from the F_1 of lake trout maternal origin. Since this effect disappears at the high acclimation temperatures, the expression or action of these modifiers is acclimation temperature dependent.

Comparing the effects of the LB ♀ F_1 and the LB ♂ F_1 by comparing the differences in resistance between crosses such as B×BL and B×LB with those between BL×B and LB×B (Table 3), it is seen that the LB F_1 egg has a greater effect than the LB F_1 sperm. Hence, there appears to be a strong cytoplasmic effect. This cytoplasmic effect is also expected to be expressed in the F_1 hybrids with the L×B F_1 being lower in resistance than the B×L F_1. However, except for the high test temperatures at 5 C acclimation (see footnote 3),

this effect was not observed and it is believed that the cytoplasmic effect is masked by the heterotic effect observed for the F_1 hybrids, discussed earlier.

Since the model was derived from variance component analysis, which is based on estimates of variances that have fairly large errors associated with them, one might suggest that other, similar models will also fit the data. It was attempted to fit a model with fewer factors to the data. A single factor model, for example, was tested against the data at 5 and 10 C acclimation and was found not to fit because the predicted phenotypic variances were significantly larger than the observed phenotypic variances.

Such tests suggest that the model described here is the simplest that can be fitted to the data. This does not imply that more complicated models involving more factors and interactions among these factors cannot be fitted to the data. In the model described here, however, simply increasing the number of factors generates distributions that do not fit the data.

The factors described do not necessarily represent individual genes; they could be segments of chromosomes, or even whole chromosomes. This analysis indicates that probably five chromosomes at the high acclimation temperatures, and two at the low acclimation temperature, are involved in determining the difference in resistance to high temperature between the lake trout and the brook trout.

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Linear Regressions in Fishery Research

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RICKER, W. E. 1973. Linear regressions in fishery research. J. Fish. Res. Board Can. 30: 409–434.

A number of regression situations in fish and fishery biology are examined, in which both of the variates are subject to error of measurement, or inherent variability, or both. For most of these situations a functional regression line is more suitable than the ordinary predictive regressions that have usually been employed, so that many estimates now in use are in some degree biased. Examples are (1) estimation of the exponent in the weight:length relationship, where almost all published values are somewhat too small; and (2) estimating the regression of logarithm of metabolic rate on log body weight of fish, where the best average figure proves to be 0.85 rather than 0.80. In the very common situation where the distribution of the variates is non-normal and open-ended, a functional regression is the most appropriate one even for purposes of prediction. Two ways of estimating the functional regression are (1) from arithmetic means of segments of the distribution, when computed symmetrically; and (2) from the geometric mean of one predictive regression and the reciprocal of the other. The GM regression gives a more accurate estimate when it is applicable; it is appropriate in all situations where the variability is mainly inherent in the material (little of it due to errors of measurement), or where the measurement variances are approximately proportional to the total variance of each variate; and it is the best estimate available for short series with moderate or large variability even when neither of these conditions applies. When error in X results solely from the measuring process the predictive regression of Y on X is also the functional regression if observations of X are not taken at random but rather have pre-established values, as is usual in experimental work. The uses of the various regressions are summarized in Table 8.

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L'auteur examine un certain nombre de régressions rencontrées en biologie des poissons et des pêches, dans des situations où les deux variables aléatoires sont sujettes à des erreurs de mesure, à une variabilité inhérente, ou aux deux. Dans la plupart de ces situations, une droite de régression fonctionnelle est plus appropriée que les régressions prédictrices ordinaires généralement employées, et qui font que plusieurs estimations présentement en usage sont biaisées jusqu'à un certain point. Comme exemples, on peut citer (1) l'estimation de l'exposant dans la relation poids-longueur, dont presque toutes les valeurs publiées sont un peu trop faibles; et (2) l'estimation de la régression du logarithme du taux métabolique en fonction du logarithme du poids du corps du poisson, dont la meilleure moyenne s'avère 0.85 plutôt que 0.80. Dans le cas très fréquent où la distribution des variables aléatoires est non normale et à extrémités ouvertes, une régression fonctionnelle est plus appropriée, même pour faire des prévisions. Deux façons d'estimer la régression fonctionnelle sont: (1) à partir de moyennes arithmétiques de segments de la distribution, calculées symétriquement, et (2) à partir de la moyenne géométrique d'une régression prédictrice et de la réciproque de l'autre. La régression basée sur cette dernière méthode, lorsque applicable, donne un estimé plus précis; elle peut s'appliquer à toutes les situations dans lesquelles la variabilité est inhérente surtout au matériel (et très peu aux erreurs de mesure), ou dans lesquelles les variances de mesure sont à peu près proportionnelles à la variance totale de chaque variable aléatoire; c'est de plus le meilleur estimé à notre portée pour de courtes séries à variabilité modérée ou grande, même si ni l'une ni l'autre de ces conditions ne sont applicables. Lorsque l'erreur en X est le résultat du seul processus de mesure, la régression prédictrice de Y en X est aussi la régression fonctionnelle si les observations de X ne sont pas prises au hasard, mais ont plutôt des valeurs préalablement établies, comme c'est ordinairement le cas dans des travaux expérimentaux. Le tableau 8 résume les usages des diverses régressions.

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THIS paper examines the applicability of estimates of parameters obtained from certain regression lines to several situations commonly encountered in fishery biology. There is a large statistical literature on the subject of regressions, of which a number of pertinent titles are listed in the References. The excuse for an amateur entering the field is that outstanding problems in fisheries mainly concern the selection and interpretation of parameters rather than their computation.

Two types of variability affect the position of regression lines: that which is caused by errors of measurement, and that which is inherent in the material being measured. I will consider natural variability first, then measurement errors, and finally combinations of the two.

A. Natural Variability only, with Normal Distributions

PREDICTIVE REGRESSIONS

1. Two variable quantities X and Y are believed to be linearly related. N pairs are measured, without appreciable error. Let x and y represent the quantities as measured from their means, i.e. $x = X - \overline{X}$; $y = Y - \overline{Y}$. Also, the standard deviations of X and Y are s_x and s_y respectively, and the linear correlation coefficient between X and Y is $r = \sum xy/(\sum x^2 \sum y^2)^{\frac{1}{2}}$. In each case we will consider the relationship in terms of a graph of Y plotted against X on rectangular coordinates (Fig. 1).

2. The *regression of Y on X* minimizes the sum of squares of *vertical* distances from the points to the line (line A in Fig. 1). Consider the equation:

$$Y = a + bX \tag{1}$$

The slope of (1), which is the *regression coefficient*, is estimated as:

$$b = \frac{\sum xy}{\sum x^2} \tag{2}$$

The Y-axis intercept, a, is estimated by passing a line of slope b through the means of the two distributions; that is:

$$Y - \overline{Y} = b(X - \overline{X}) \tag{3}$$

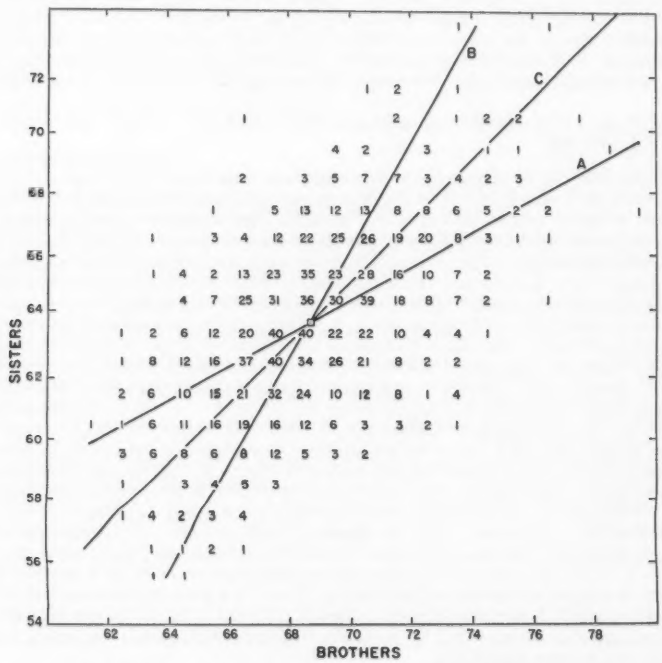

FIG. 1. Distribution of adult heights in inches of 1379 sisters and brothers, and computed regressions. Data from Table 64 of Pearson and Lee (1902), rounded to the nearest integer.

From this are derived two convenient expressions for a:

$$a = \overline{Y} - b\overline{X} = \frac{\sum Y - b\sum X}{N} \quad (4)$$

The expressions in (4) can be used to determine the Y-axis intercept in all of the methods to follow, using the appropriate slope in each case.

The X-axis intercept of the line (1) is equal to $-a/b$. This point is often useful, along with $(\overline{X}, \overline{Y})$, for plotting the position of a line when the Y-axis intercept lies outside the range of values on the graph.

3. The variance of the observations from the above regression line in the vertical direction can be expressed in a number of ways, three of which are:

$$s^2_{yx} = \frac{\sum y^2 - (\sum xy)^2/\sum x^2}{N-2} = \frac{\sum y^2 - b\sum xy}{N-2}$$

$$= \frac{\sum y^2 (1 - r^2)}{N-2} \quad (5)$$

Two forms of the variance of the regression coefficient b are:

$$s^2_b = \frac{s^2_{yx}}{\sum x^2} = \frac{y^2 (1 - r^2)}{N-2} \quad (6)$$

where $y^2 = \sum y^2/\sum x^2$ — a statistic employed in Section 8 below. Using N – 2 df and Student's distribution, the square root of (6) can be used to set confidence limits for the position of the regression line, or to compare the regression with any fixed value. Comparing it with 0 means computing b/s_b, which is a test of the reality or "significance" of the association between X and Y. This test can also be made more simply by computing the correlation coefficient r and checking it against a table of significant levels of r, using N – 2 df.

However, confidence limits for the position of the regression line do not constitute confidence limits for estimates of Y computed from it. Suppose we estimate the value of Y that corresponds to X = P by calculation from (1). There are two situations. The computed Y can be regarded either as an estimate of the *mean* value of Y at X = P, or as an estimate of a *single* value of Y corresponding to one observation of X = P. For the mean the variance is:

$$s^2_{yx}\left(\frac{1}{N} + \frac{(P - \overline{X})^2}{\sum x^2}\right) = s^2_{yx}/N + s^2_b (P - \overline{X})^2 \quad (7)$$

The first term of (7) is the variance of the mean value of Y that corresponds to the mean of X; the second term is the additional variance, due to uncertainty in the position of the regression line, when Y is computed from a value of X other than $\overline{X}$. As a special case of (7), the Y-axis intercept, a, of the line (1) has a variance of:

$$s^2_a = s^2_{yx}\left(\frac{1}{N} + \frac{X^2}{\sum x^2}\right) \quad (8)$$

Finally, the variance of any single estimate of Y made from (1) is:

$$s^2_{yx}\left(1 + \frac{1}{N} + \frac{(P - \overline{X})^2}{\sum x^2}\right) \quad (9)$$

where P is the value of X for which an estimate of Y is being made. Here the variance about the regression line, s^2_{yx}, is added to (7) in order to obtain the total variance of a single estimate.

The above expressions are described, in one form or another, in practically all handbooks of statistics, for example Snedecor (1946, Section 6.9) or Sokal and Rohlf (1969, Section 14.5), and small desk-top electronic computers are routinely programmed to get as far as expression (5) or (6).

4. The *regression of X on Y* minimizes the sum of the squares of *horizontal* distances from the points to the line, on a graph of Y against X (line B in Fig. 1). This regression is usually written in the form:

$$X = c + dY \quad (10)$$

where:

$$d = \frac{\sum xy}{\sum y^2} = \frac{r^2}{b} \quad (11)$$

The other statistics above can be obtained, for this line, by interchanging x and y, X and Y, in expressions (4)–(9).

For plotting on a graph with Y on the ordinate it is sometimes convenient to rearrange (10) as:

$$Y = -\frac{c}{d} + \frac{X}{d} \quad (12)$$

5. Predictive relationships are used to estimate Y when X is known, or X when Y is known. The regression of Y on X is used to estimate Y from X, and the regression of X on Y is used to estimate X from Y. When the two predictive regression lines are plotted on a graph of the usual sort (Y on the ordinate, X on the abscissa), it turns out that the regression of X on Y always has more slant (that is, a steeper slope, ignoring the sign) than the regression of Y on X (Fig. 1). The difference in slope may be small or large — varying from 90° when there is no correlation between the variates ($r = 0$) to zero when correlation is perfect ($r = \pm 1$). In other words, if the data points lie close to the regression lines, the difference between the slopes of the two regressions is small; if they are widely scattered, b and $1/d$ differ substantially. An increase in number of observations does not affect the magnitude of estimates of b and d *systematically*, as long as the same population is being randomly sampled, though of course their reliability improves.

When both X and Y have a normal or Gaussian distribution, the array is said to be bivariate normal. In that event the points when plotted tend to lie in an area bounded by an ellipse (*Jones 1937, fig. 1; Teissier 1948, fig. 4*). The major axis of this ellipse is the line described by expression (19) below.

6. As an illustration of the use of predictive regressions, the distribution of adult heights of brothers (X) and sisters (Y) is plotted in Fig. 1. This is a bivariate normal distribution or close to it. The basic statistics of the series are:

$$\bar{X} = 68.678 \text{ inches}; \quad \bar{Y} = 63.809 \text{ inches}; \quad N = 1379$$
$$\sum x^2 = 9510; \quad \sum y^2 = 9267; \quad \sum xy = 5180;$$
$$b = 0.5447 \quad d = 0.5590 \quad r = 0.5518$$

The regression coefficients b and d are from (2) and (11), and the correlation coefficient is their geometric mean. Applying (4), we obtain the predictive equations:

$$Y = 26.4 + 0.545X \tag{13}$$

$$X = 33.0 + 0.559Y \tag{14}$$

These lines are plotted as A and B in Fig. 1. If we wish to estimate a sister's height, knowing that of one of her brothers, we use (13). Similarly to estimate a brother's height, knowing his sister's, we use (14). From Fig. 1 it is easy to see why two regression lines are necessary for these predictions: the regression of Y on X runs through the approximate middle of each vertical array of frequencies, and similarly the regression of X on Y runs through the middle of each horizontal array. Thus in addition to their practical use for purpose of estimation, the predictive regressions also help to describe the normal bivariate relationship.

7. The standard error of a predicted height can be obtained from (9). For example, from (13) we calculate that a woman whose brother is 72 inches tall will most probably be 65.6 inches tall. To obtain the standard error, we first compute the variance from the regression line from (5) as:

$$s^2_{yx} = \frac{9267 - (5180)^2/9510}{1377} = 4.681$$

For inserting in (9) we have also:

$$N = 1379$$
$$P - \bar{X} = 72 - 68.678 = 3.322$$
$$\sum x^2 = 9510$$

The variance of the estimated height is then:

$$4.681\left(1 + \frac{1}{1379} + \frac{3.322^2}{9510}\right) = 4.690$$

Accordingly the standard error of the estimate 65.6 inches is the square root of this; i.e. 2.17 inches.

The estimated *mean* height of women having a brother 72 inches tall is also 65.6 inches, but the standard error of this mean is much less; from (7) it is the square root of:

$$4.681\left(\frac{1}{1379} + \frac{3.322^2}{9510}\right) = 0.00883$$

which is 0.0940 inch.

Functional Regressions

8. Sometimes we need a measure of the central trend of a natural distribution, or what is often called the functional regression. In the example of Fig. 1 it would be the line defining a series of corresponding heights of sisters and brothers that best represents the reciprocal relation between the two. Let the equation of this line be:

$$Y = u + vX \tag{15}$$

As noted above, the two predictive regressions minimize the sum of the squares of the vertical and horizontal distances, respectively, from the points to the regression line. To obtain a central value Teissier (1948) suggested applying the same principle by finding the line which minimizes *the sum of the products of the vertical and horizontal distance* of each point from the line. He showed that the slope of this line is:

$$v = \pm\sqrt{\frac{\sum y^2}{\sum x^2}} \tag{16}$$

Notice that (16) is based solely on the sums of squares of the deviations, without involving any cross-products. By comparing formulae four other forms of this relationship can readily be derived, any one of which may prove useful depending on what statistics are immediately at hand:

$$v = \pm\sqrt{\frac{b}{d}} = \pm\frac{s_y}{s_x} = \pm\frac{b}{r}$$
$$= \pm[b^2 + s_b^2(N-2)]^{\frac{1}{2}} \tag{17}$$

The first of these shows that v is the geometric mean of the predictive regression of Y on X and the reciprocal of the predictive regression of X on Y. The sign of v is of course the same as the sign of b, d, and r.

I will refer to v as the *geometric mean estimate of the functional regression of Y on X*, or more briefly, the GM regression. The GM regression of X on Y is exactly the same line as described by (16), but its slope is equal to $1/v$ because the axes are reversed.

Since the slope of the GM regression line is determined solely by the two standard deviations, then no matter how the variates X and Y are paired up, their GM regression can have only two values, numerically the same but differing in sign. The regressions of Y on X and X on Y approach or diverge from these two lines as the

degree of association between X and Y waxes or wanes. For this reason it is impossible to use the GM regression to test the reality of the association between two sets of variates; that is, v/s_v cannot be used to test significance in the way that b/s_b is used.

9. The variance of a GM regression, s_v^2, is the same as that of the corresponding predictive regression (Teissier 1948; Kermack and Haldane 1950); that is, it is expression (6) above. Strictly speaking this should not be used to estimate symmetrical confidence limits for v in the usual way, because the GM line is not situated symmetrically with respect to the Y variates when viewed in the vertical direction (nor with respect to the X variates when viewed horizontally). I have not found any satisfactory statistical treatment that supplies the necessary asymmetrical limits. Figure 2 illustrates the actual distribution of GM regressions obtained by repeated random sampling from the distribution of Figure 1[1]. The bottom panel shows 100 estimates of v obtained from samples of 11 pairs of variates each, and the top panel shows 150 samples of 25 pairs each. The distributions are both asymmetrical, although for the larger N this is not immediately obvious. The broken lines in Fig. 2 show the mean of the parent distribution and the symmetrical 95% confidence limits for the two sizes of sample. The latter were computed, using expression (6), as:

$$v \pm t\left(\frac{v^2(1-r^2)}{N-2}\right)^{\frac{1}{2}}$$

where v and r are given their values for the parent distribution ($v = 0.987$, $r = 0.552$), N is the sample size in question (11 or 25), and t is the Student multiplier for 95% confidence with N–2 df. It turns out that 96% of the 11-item values lie within the 95% confidence interval, as do 94.7% of the 25-item values — a result which increases our confidence that expression (6) does in fact represent the variance of the GM regression (Kermack and Haldane (1950) found this "rather surprising").

In addition, 95% confidence limits were computed for each individual v using the estimate of s_v obtained from its own series of 11 or 25 pairs of variates. It was found that 96% of the 11-variate series included the true value 0.987 in the symmetrical 95% confidence interval on either side of the estimated v, and 93.5% of the 25-variate series did so.

Thus it is possible to recommend using ordinary symmetrical confidence limits for the GM regression. They are a reasonable approximation to the true limits and will rarely lead to incorrect conclusions.[2]

10. Another name that has been used for the GM regression is the "reduced major axis" (Kermack and Haldane 1950), while Teissier (1948) called it "la relation d'allométrie." Jones (1937) and the above authors compare the GM regression with other regressions; see especially Teissier's fig. 4.

As far as I know, the GM regression has not previously been recommended as the best estimate of the functional regression for all cases in which the variability of both variates is wholly natural. (Even Teissier, while recommending it generally for morphometric work, was prepared to admit possible exceptions.) However, in this situation there seems no way, even conceptually, of separating the variable element in the position of a given point into two components, one due to variation in Y at a given X and one due to variation in X at a given Y. Thus the whole idea of two "point variances" and their ratio λ, used in Section 29 below, becomes meaningless here.[3]

In morphometric work, for example, the logarithm of one body measurement is compared with the logarithm of another. Teissier uses the example of the length and breadth of a crab's carapace, and obviously a carapace that is relatively broad can also be described as relatively short; the two effects cannot be disentangled. This becomes somewhat less obvious in the case of, for example, a comparison between the length of a particular fin on a fish and its body length; but it is just as true to say that the fish is long in relation to the fin as that the fin is short relative to the fish — operationally there is no difference. In the same way, if a fish is "heavy for its length," it makes no sense to argue whether it is obese or whether it is dwarfed.

However, the GM regression's usefulness extends far beyond the field of allometry. In Fig. 1, for example, each point represents a pair of observations to which both the brother and the sister contribute natural variability, and there is no way of separating their individual contributions. Since the measurement error is insignificant by comparison, the GM regression is the appropriate measure of the functional relationship. The same is true

[1] Dr J. R. Calaprice suggested and carried out these computations on a small computer, in cooperation with Mr L. J. Carlsen.

[2] The confidence limits computed from formulae (15)–(19) of Jolicoeur and Heusner (1971), based on Creasy's (1956) results, give wider limits than those indicated by the random sampling of Fig. 2. When $\lambda = \sum y^2/\sum x^2$ the limits in expressions (15) and (17) of Jolicoeur and Heusner reduce to:

$$v\left[\frac{\sum xy - q}{\sum xy + q}\right]^{\frac{1}{2}};\ v\left[\frac{\sum xy + q}{\sum xy - q}\right]^{\frac{1}{2}}$$

where: $$q^2 = \frac{t^2[\sum y^2 \sum x^2 - (\sum xy)^2]}{N-2}$$

t being the Student multiplier for N-2 df at the chosen level of significance. This formula puts the 95% limits for N = 25 in Fig. 2 at 0.45 and 2.15 — a much greater range than is indicated by the sampling. When N = 11 it turns out that $q > \sum xy$, so no answer is possible.

[3] If in spite of this we try to estimate separate variances for X and Y by using deviations from the two predictive regression lines, it is shown in Section 30 that this leads to expression (16).

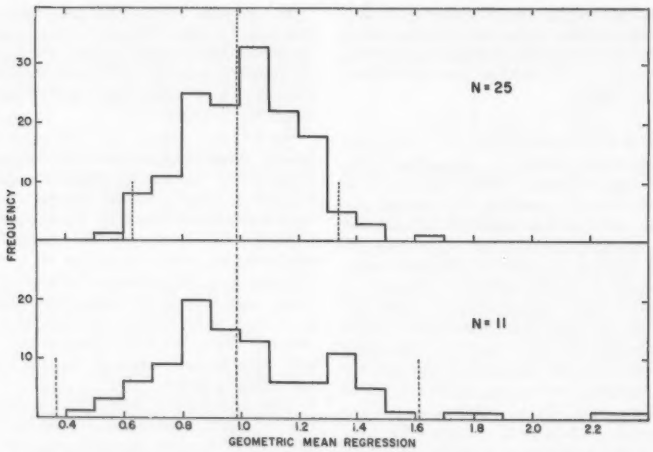

FIG. 2. Distribution of estimates of the GM regression in samples drawn randomly from Fig. 1. *Top*: 150 samples of 25 pairs each. *Bottom*: 100 samples of 11 pairs each.

of any other comparison in which the sources of variability in the measurements of both items are almost wholly natural, in the sense of not being a part of the measuring process and hence not subject to partition into separate moieties. Additional examples are given in Sections 42–55 below.

11. For a numerical example, from the data of Fig. 1 given in Section 6 the GM regression is estimated from (16) as:

$$v = \sqrt{\frac{9267}{9510}} = 0.9871$$

The Y-axis intercept is $u = 63.809 - 0.9871 \times 68.678 = -3.98$ inches. This is shown as line C in Fig. 1.

The standard error of v is the same as that for b; hence from (6):

$$s_v = s_b = \left(\frac{4.681}{9510}\right)^{\frac{1}{2}} = 0.0222$$

Approximate 95% confidence limits, with N – 2 = 1377 df, are obtained from:

$$0.987 \pm 1.960 \times 0.0222 = 0.944 \text{ and } 1.030$$

12. Teissier (1948, p. 32) gives an expression for computing the variance of an estimate of Y made from the GM regression corresponding to an observed X = P. Modified by using N – 1 instead of N in the first term, this can be written:

$$\frac{\sum y^2 (1 - r^2)}{N - 1} + v^2 (1 - r)^2 (P - \bar{X})^2 \qquad (18)$$

This expression is comparable to expression (9) for the predictive regression, but again no method of computing accurate confidence limits appears to have been developed. so the symmetrical approximation must be used. Variances calculated from (18) are greater than those from (9) because the GM line is a poorer fit than b is to the observed points *in the vertical direction.*

MAJOR AXIS

13. Although it should not be called a regression line, it is of some interest to know how to compute the major axis of the correlation ellipse. This is the straight line that minimizes the sum of the squares of the distances from the observed points to the line in a direction at right angles to the line, *when one unit of measurement occupies the same absolute distance on the X and Y coordinates.*

The slope of the major axis, z, was discovered in the last century by R. J. Adcock (Kermack and Haldane 1950) and C. H. Kummell (Deming 1943); it has the equation:

$$z = \frac{\sum y^2 - \sum x^2 + [(\sum x^2 - \sum y^2)^2 + 4(\sum xy)^2]^{\frac{1}{2}}}{2\sum xy} \qquad (19)$$

Karl Pearson (1901) arrived at the same result in a different form:

$$z = \tan \theta, \text{ where } \tan 2\theta = \frac{2\sum xy}{\sum x^2 - \sum y^2} \qquad (20)$$

Still another expression for the slope of this line is given by Sokal and Rohlf (1969, p. 527); in our symbols it is:

$$z = \frac{\sum xy}{\lambda_1 (N-1) - \sum y^2} \qquad (21)$$

where:

$$2\lambda_1 (N-1) = \sum y^2 + \sum x^2 + [(\sum y^2 + \sum x^2)^2 - 4(\sum y^2 \sum x^2 - (\sum xy)^2)]^{\frac{1}{2}} \qquad (22)$$

Sokal and Rohlf (1969) also (p. 529, item 5) illustrate Anderson's (1963) method of computing confidence limits for z, although strictly speaking it applies only to large samples.[4]

The statistic λ_1 defined by (22) is known as the first eigenvalue or first latent root. The second eigenvalue, λ_2, is found from:

$$\lambda_2(N-1) = \sum y^2 + \sum x^2 - \lambda_1(N-1) \qquad (23)$$

The two eigenvalues establish the shape of the correlation ellipse: the ratio of the major axis to the minor axis is equal to λ_1/λ_2.

14. For the data of Fig. 1, the slope of the major axis is, from (19):

$$z = \frac{9267 - 9510 + [(9267 - 9510)^2 + 4 \times 5180^2]^{\frac{1}{2}}}{2 \times 5180} = 0.977$$

The Y-axis intercept is $63.809 - 0.977 \times 68.678 = -3.28$ inches. In this example the major axis lies very close to the GM regression (0.987), so close that it would be difficult to draw them separately on Fig. 1.

To establish the first eigenvalue we have from (22):

$2\lambda_1 (N-1) = 9267 + 9510 + [(9267 + 9510)^2 - 4(9267 \times 9510 - 5180^2)]^{\frac{1}{2}}$

$\lambda_1 (N-1) = 14570$

Hence from (21), $z = 5180/(14570 - 9267) = 0.977$, as before.

Also, from (23):

$$\lambda_2 (N-1) = 9267 + 9510 - 14570 = 4207$$

The ratio of the axes of the correlation ellipse is therefore $14570/4207 = 3.46$.

15. In Section 8 we noted that, in order to obtain a central trend line for a bivariate distribution, Teissier had minimized the sum of products of deviations in the vertical and horizontal directions. A natural question arises, why not use the major axis for this purpose, since it minimizes the sums of squares of the shortest distances from the observed points to the line? As a matter of fact the early investigators developed the line partly for this purpose, and it has been so used up to very recently, by some authors.

Unfortunately, as Jones (1937) and others have pointed out, the particular units of measurement that are used affect the major axis differently from the GM and the predictive regressions. For example, suppose that in Fig. 1 the women had been measured in half-inches and the men in inches. In that event the predictive and GM regressions of Y on X are both numerically twice as great as computed above, and the predictive regression of X on Y is half as great. However the slope of the major axis becomes 2.99, which isn't any simple multiple of the original figure 0.977. Thus if the new data were to be plotted exactly as in Fig. 1 by doubling the figures on the ordinate scale, the position of lines A, B and C would not change, but the major axis would no longer practically coincide with the geometric mean line C; rather it would lie about midway between lines B and C. If the sisters are measured in still smaller units, the major axis becomes even steeper, and in the limit it coincides with line B, the predictive regression of X on Y.

Thus the position of the major axis can be made to lie anywhere between the two predictive regressions, simply by changing the units of measurement. Since measurement units can be chosen quite arbitrarily, obviously the major axis is not a good statistic for characterizing the trend of a bivariate distribution. A functional regression line is much preferable, since its position is independent of the units of measurement employed.

The above results can also be derived from the form of expressions (19)–(21). These require that X and Y be in the same units (since $\sum x^2$ is subtracted from $\sum y^2$), and when this is so they define a dimensionless quantity. Thus the major axis can have only a formal geometrical significance. The predictive and the GM regression of Y on X, by contrast, have the dimensions of Y/X, as is obviously necessary for a line that describes the change in Y per unit change in X.

B. Natural Variability and Non-Normal Distributions

16. The theoretical development of predictive and functional regressions has mainly been in terms of data where both variates are normally distributed, or are reasonably close to it — situations that we may identify as *Type A*. However regression lines are continually being computed for all sorts of non-normal distributions and other situations that are atypical from the statistical point of view, usually without much consideration of the consequences. Some of the situations that occur more frequently can be classified as follows.

Type B — There is a concentration of observations near one end of the frequency distribution, and progressively fewer at the other end; or there may be high and low points in the distribution, or even gaps. An example is the distribution of lengths and weights in a fish population when all ages are sampled in proportion to their abundance.

[4] Mr. Robin Allen pointed this out to the author. He also notes that the expression for H in Sokal and Rohlf 1969 (p. 529) should have n–1 instead of n in the denominator.

Type C — Observations are more or less equally frequent throughout the entire range of the distribution. This might occur naturally, but usually it is a result of conscious selection of observations from a parent distribution that may be normal or of various other shapes, or which may not have any objective existence.

Type D — A regression line computed from one distribution is used to convert observations (of the same general type) drawn from a different distribution.

Type E — The distribution of observations available is not a random sample of the parent population. The latter may not have any objective existence, but if it does, it is either unknown or not pertinent to the regression situation under consideration, or both.

Type F — The Y and X variates are averaged within equal short intervals of X, and a regression line fitted to the means with uniform weighting. (Sometimes intervals with very few variates are rejected because of their large sampling error.) This procedure may be adopted because of a desire to give equal weight to the whole range of observations available, particularly when there is doubt about the exact linearity of the relationship. Sometimes, however, it has been done merely to save time.

An important problem that can arise with any of the situations B through E above is that of homogeneity of variance. (It does not arise with type A because a bivariate normal distribution implies homogeneous variance.) Regression theory assumes that the variance of Y about the predictive regression line is the same everywhere, i.e. it is independent of the value of X. While small departures from this condition are not fatal, any serious departure poses real problems. Sometimes homogeneity of variance can be obtained by transforming one or both variates, and in biology logarithmic transformations are among the most common. However it needs to be remembered that any transformation will change the nature of the relationship between the variates. For example, if Y and X are linearly related, the transformed variates will not necessarily be so. Ideally a transformation should both stabilize variance and introduce (or retain) linearity, and it is fortunate that in a number of important situations this can be managed. In situations of Type F, however, homogeneity of variance is deliberately destroyed whenever there are more observations in one part of the X range than in another.

Type B Situations

17. No general rules can be given for handling naturally asymmetrical distributions; when one actually occurs, some effort should be made to determine its characteristics from the regression point of view. It may turn out that the usual predictive and functional regressions will be adequate for their respective uses, or at least be the best mechanism available.

Figure 3 is an example of a bivariate normal distribution cut off at a given X value — in this case at 69 inches. If we were to consider the truncated distribution as an entity in itself, the three regressions drawn — Y on X, X on Y, and functional — will serve their respective functions fairly well. On the other hand, if we attempt to estimate the characteristics of the parent distribution from this one, a comparison of Fig. 3 with Fig. 1 shows that only the regression of Y on X has any pertinence.

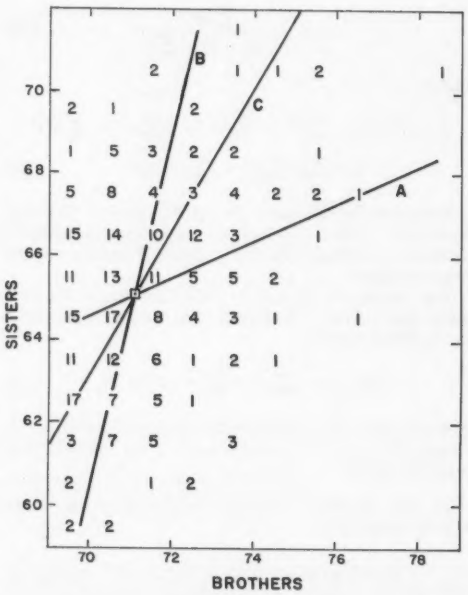

FIG. 3. Diagram of Fig. 1 truncated at brothers of 69 inches and with observations reduced to half by random selection. The two predictive regression lines and the geometric mean line are shown.

Alternatively, if the parent distribution had been bisected at a given Y value, then the regression of X on Y can estimate the same statistic in the parent distribution, but no other regression will be useful.

Similarly, if the parent distribution is divided in a plane at right angles to the trend, the GM regression line can estimate the functional trend of the parent distribution, but neither predictive regression will be applicable to the latter. This is shown in Table 1: the predictive regressions for each of the two symmetrical halves of Fig. 1 are much smaller than those for the whole distribution, whereas the GM regressions agree within 4–6% — well within limits of sampling fluctuation.

Type C Situations

18. Very frequently we encounter situations in which it is desirable to examine as broad a range of information as possible, yet random sampling will give only a few observations at the extremes of the distribution along with an unmanageable superfluity in between. Or there may be no objective distribution from which to sample,

TABLE 1. Correlation and regressions for the distribution of Fig. 1 and for each of two segments obtained by dividing it approximately into halves transversely to the trend (i.e. by a line through $(\overline{X}, \overline{Y})$ and perpendicular to the GM regression). Statistics of the daughter distributions that resemble the parental are marked by an asterisk.

	N	Correlation r	Regressions: Y on X b	Regressions: X on Y d	Regressions: GM v
Whole distribution	1379	0.552	0.545	0.559	0.987
Lower portion	702	0.140	0.147	0.133	1.050*
Upper portion	677	0.108	0.103	0.114	0.945*

so that the kind of distribution used must be determined by the investigator. In such situations a common practice is to limit the number of Y observations to a fixed number in each short interval of X, but use all data that are available in intervals that have less than this limit.

Figure 4 shows such a selection taken randomly from Fig. 1: at each inch of the brothers' heights five pairs of measurements were chosen, or whatever smaller number was available. Equally, such a selection can be made on the basis of the sisters' heights.

In the B and C panels of Table 2 two selections of each sort are compared. When the number of Y observations is limited at each X, the predictive regression of Y on X is a reasonable estimate of the same statistic in the larger distribution. Similarly when the X observations are limited, the predictive regression of X on Y corresponds fairly well to the parental X on Y.

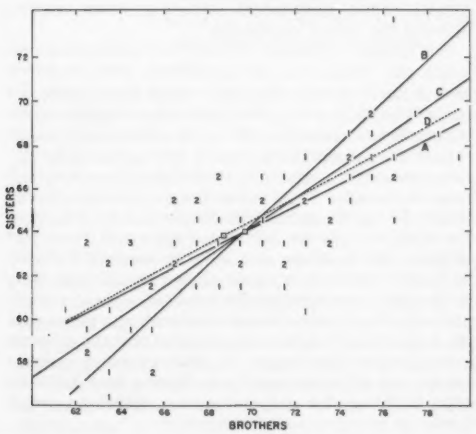

FIG. 4. Diagram of Fig. 1 reduced by limiting each × category to 5 observations chosen randomly. A: regression of Y on X; B: regression of X on Y; C: GM regression; D: the regression of Y on X for the parent distribution.

19. Neither of the above procedures will provide an unbiased estimate of the functional regression. To obtain the functional regression in this situation a series can be made up by selecting half of the pairs of variates on the basis of X intervals and half on the basis of Y intervals, as shown in panel D of Table 2. A procedure that will usually be easier is to take a geometric mean from two predictive regressions that are based on approximately the same number of observations, each obtained from a series chosen in the appropriate way. For example, in Table 2 the GM of 0.526 and 1/0.563 is 0.967 and the GM of 0.594 and 1/0.587 is 1.006; both are acceptable estimates of the parental functional regression 0.987.

In situations where only part of the distribution is available because of some kind of *natural* selection process, the selection is usually neither abrupt as in Fig. 3, nor related solely to the Y or the X category as in Fig. 4. Rather it tends to fade away at each end and to be symmetrical with respect to both variates. As a result, the functional regression estimate is not biased. The predictive regressions, however, *are* biased, creating the Type E situations described below.

TYPE D SITUATIONS

20. Statisticians assume that a predictive regression will be used only to convert items drawn from the same distribution as that which supplied the regression. Occasionally this is made explicit, for example by Lindley (1947, p. 234): "it is understood that the x_1 [our X] from which x_2 [Y] is to be predicted comes from the same population as the x_1's used in the estimation of the regression line." Notice that it is not enough that the X-values to be used for prediction lie within the same range as those used to estimate the regression line. Rather they must belong to the identical population, otherwise the estimates obtained will be biased (since any different population would produce a different regression line).

However biologists frequently make a practice of applying a predictive regression line to other material

TABLE 2. Comparison of the bivariate normal distribution of Fig. 1 with 6 randomly-drawn daughter distributions. Statistics of the daughter distributions that resemble the parental are marked by an asterisk.

	N	Regressions		
		Y on X *b*	X on Y *d*	GM *v*
A. Parental distribution	1379	0.545	0.559	0.987
B. Y values limited to 5 at each X				
First trial	79	0.526*	1.055	0.707
Second trial	79	0.594*	0.965	0.785
C. X values limited to 5 at each Y				
First trial	83	1.134	0.563*	1.420
Second trial	83	1.069	0.587*	1.350
D. Up to three Y values selected at each X and up to three X values at each Y				
First trial	98	0.754	0.718	1.024*
Second trial	98	0.715	0.731	0.989*

of a similar sort, in which the distributions of frequencies may be quite different from the original distribution. Sometimes they will even extrapolate it somewhat to include a range of values not present in the original distribution — always a risky practice, but sometimes unavoidable.

It is difficult to give general rules for this situation. If the new material is very similar to the old, the old predictive regression may work well enough. If the new material is dissimilar, and particularly if it involves extrapolation, the old line will give biased estimates, not merely in the extrapolated region but throughout the whole range. In such cases the situation often approaches Type E below, and the GM regression may become the best basis for prediction, because it is the one toward which the predictive regressions converge as range increases indefinitely.

TYPE E SITUATIONS (*open-ended distributions*)

21. A common type of regression situation is where the *population* of measurements under consideration has no objective frequency distribution, so that its shape must be determined or imagined by the investigator. The obvious population to choose, in most instances, is one in which measurements are equally dense at successive equal intervals along the trend line, and are arranged symmetrically with respect to the latter. The *sample* available may or may not have a similar distribution. This can be arranged if the sampling is done by the investigator, and the considerations of Sections (17)–(19) will apply. Natural samples rarely include the whole range of possible values, but fortunately they are usually symmetrical with respect to the trend, and this can be assumed unless there are obvious indications to the contrary.

An even commoner situation, very similar to the above, is where a natural population exists but its shape is unknown or poorly known, or it is variable, or it is not really pertinent to the study being made. The sample available has not been drawn randomly from the population, and frequently half or more of the range of the population is not available for sampling by the method used. In these situations too it is usually best to consider the material available as a non-random symmetrical sample from a hypothetical evenly-distributed original, ignoring the actual population.

For example, consider a group of observations on length and weight in a fish population, from which we want a line to use in estimating weight from length. To relate this sample to any distribution that exists in nature is generally not possible, and in any event would be unrewarding. For one thing, usually only a part of the full range of lengths and weights is available. Even if the full range is present, it is not desirable that the sample should reflect the actual population distribution of fish sizes: the latter will change with the seasons and from year to year, and its shape at a given moment will usually be known only in a very general way, though it is likely to be highly asymmetrical. Thus the only way to achieve generality for a weight:length relationship is to consider the data as a non-random but symmetrical sample from a hypothetical distribution of observations of uniform density and of indefinite extent. Similar considerations apply to many other natural regression situations, which I refer to as "open-ended" situations.

22. When only a portion of the total range of actual or potential data is available in a sample from an open-ended distribution, a predictive regression tends to increase in magnitude (disregarding the sign) as the range of variates sampled increases. The functional

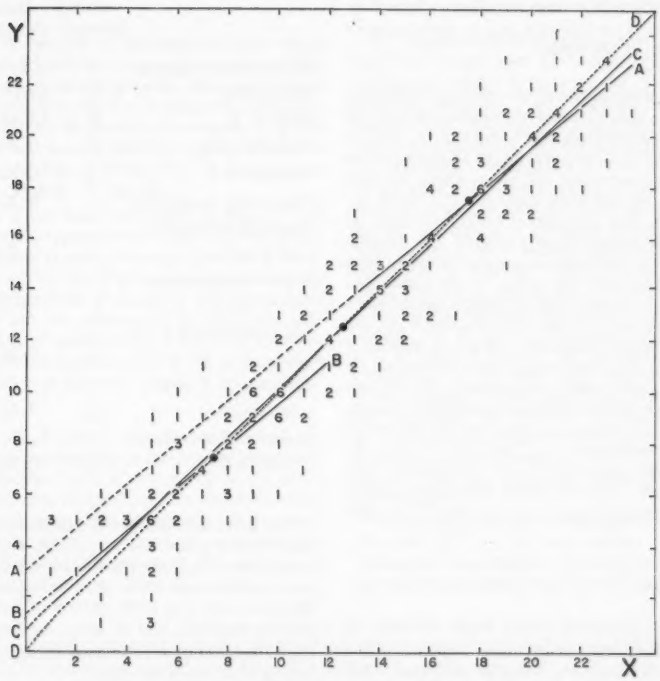

FIG. 5. Artificially randomized distribution of 240 pairs of variates, symmetrical with respect to X and Y. Dots are the means of the whole distribution and of its two halves. A: regression of Y on X for the upper half of the distribution; B: the same for the lower half; C: the same for the whole distribution; D: functional relationship between Y and X.

regression, however, does not change systematically with change in the range. Table 1 can serve as a first illustration of this, if for the purpose of the illustration we imagine that Fig. 1 is a non-random sample from an open-ended distribution. Table 1 shows that each of the two symmetrical halves of the Fig. 1 sample has a much smaller predictive regression than does Fig. 1 as a whole, while the GM regressions do not differ significantly.

With samples of small or moderate size, sampling variability can at times destroy symmetry sufficiently to reverse the expected relationship. For example, the predictive regression of Y on X for the lower half of Fig. 4 is *greater* than that for the whole distribution. But among a number of distributions studied from this point of view, a large majority of the regressions for segments of the distribution were less than for the whole of the distribution.

To get some idea of what is to be expected quantitatively, a non-normal artificial series has been prepared that is completely symmetrical with respect to the functional regression line (Fig. 5). Random digits from 0 to 4 were added to both members of each of 6 pairs of sequences of integers from 1 to 20; to make the distribution symmetrical and so eliminate sampling fluctuation, X and Y were then interchanged to provide 120 more points. The distribution was then divided into two halves transversely to the trend. Lines A and B in Figure 5 are the predictive regressions of Y on X for the two halves of the distribution, while line C is for the whole. To estimate Y, knowing X, we would use line C, according to conventional regression practice. But if we in fact had only the first half of the distribution, we would use line B, and our estimates of Y would be biased: they tend to be too small for X greater than 5, and too large for X less than 5. On the whole they are somewhat too small, because the mean of the half lies below the overall predictive regression line. Similarly, estimates made from a predictive regression for the second half will on the average tend to be too great. Notice that the overall predictive regression line cannot pass through the mean points of the two halves of this distribution: expression (30) below indicates that these means must lie on the functional regression line, as in fact they do in Fig. 5.

Summarizing, *a predictive regression of Y on X tends to have a steeper inclination to the X axis, whether positive or negative, the greater the range of points used in estimat-*

ing it; always provided that when a series is divided or truncated (naturally or artificially) this is done symmetrically with respect to the trend.

23. If an open-ended distribution extends indefinitely at one or both ends, and progressively longer symmetrical segments of it are examined, what happens to the regression statistics? The above and other examples show that the *functional regression* will vary about its true value but there is no expected trend toward either increase or decrease. The *correlation coefficient* tends to increase or decrease asymptotically toward ± 1. Finally, the slopes of the two *predictive regressions* tend to move asymptotically toward the true value of the functional regression. In Fig. 5, for example, doubling the range of points used increased b from 0.82 to 0.95; this is already close to the functional regression, which is exactly 1 because of the way the distribution was constructed. It follows that *with open-ended non-normal distributions the best regression for prediction is the functional regression, no matter how long or short a segment of the population is available for examination, and no matter what the shape of the sample, provided it is symmetrical with respect to the trend.* The functional regression should be used for predicting Y from X, or X from Y, in such circumstances.

TYPE F SITUATIONS

24. This type of situation occurs when variates are averaged within short equal intervals of X and a line is fitted with uniform weighting (except that cells with very small frequencies are often disregarded). In the case where there is a concentration of frequencies somewhere near the middle of the original distribution the overall *variance of X* will be increased by this procedure, because extreme values now have the same weight as central ones. The overall *variance of Y*, however, may not change much, for although the extremes have the same weight as the middle they have become less extreme because of the averaging process (particularly if X-intervals with very small frequencies have been rejected). The *correlation coefficient* increases greatly because of the closer fit of the Y values to the regression line, but its "significance" may change little because most of the degrees of freedom have been lost. The predictive *regression of Y on X* is usually very similar to the original regression; it differs only because of the new weighting, and there is no expected trend toward either increase or decrease. The *regression of X on Y*, however, becomes much less steep (on a graph of Y against X). The mean *variance of Y from the predictive regression line* of Y on X is greatly reduced, and it becomes non-homogeneous (it is less where the observations averaged were more numerous). Finally, the *GM regression of Y on X* moves a long way toward the predictive regression of Y on X, and loses all its usefulness as a descriptive or predictive statistic.

These changes are illustrated in the schedule below for the data of Fig. 1. (In computing the averaged distribution, X intervals with only a single observation were ignored; the next-smallest frequency is 5).

	Symbol	Original distribution	Averaged distribution
No. of pairs of variates	N	1379	15
Mean of X	$\overline{X}$	68.68	69.50
Mean of Y	$\overline{Y}$	63.81	64.27
Variance of X	s_x^2	6.90	20.00
Variance of Y	s_y^2	6.72	6.51
Correlation coefficient	r	0.552	0.989
Predictive regression of Y on X	b	0.545	0.564
Predictive regression of X on Y	d	0.559	1.735
GM regression of Y on X	v	0.987	0.570
Variance of Y from regression	s_{yx}^2	4.68	0.148
Variance of regression coefficient	s_b^2	0.000492	0.000528

The disadvantages of averaging are evidently considerable, but it would be an overstatement to say that it should never be done. For example, it can help in identifying possible curvature in the relationship. The main thing is to have a clear picture of what happens to the various statistics, and to avoid making improper use of them. In particular, functional regressions from averaged data should never be used.

C. Variability in Measuring

25. We consider now the variability or "error" that arises from the measuring process, because of imperfection of the instruments or of the observers. The simplest situation is where *all* the variability of a set of observations is of this sort, and none is natural. This situation can arise in making observations on the "ideal" relationships of physics or chemistry, for example the relation between the temperature and pressure of a given volume of gas. In biology a commoner situation is where only the X variable lacks natural variability, while the Y variable is subject to natural variability and may have significant observational error as well. An example would be observations on a fish's swimming speed (Y) in relation to temperature (X).

For the X variable in these situations there are three possibilities: (1) it may be without significant error of any sort; (2) it may be subject to measurement error but the observations are controlled (as defined in Section 27); or (3) it may be subject to measurement error and the observations are not controlled, i.e. random. For situations (1) and (2) the Y variate may have natural variability as well as (or instead of) measurement error, and the regression analysis will be the same; for situation (3) a distinction must be made between the two sources of variability in Y.

X Measured Without Error

26. When the X variable is measured without error (and lacks natural variability) it is usually considered that there is only one meaningful regression, the regression of Y on X, and the best estimate of it is given by expression (2) (Eisenhart 1939). In Section 29 it is shown that this is a functional as well as a predictive regression: to be used either for computing Y from X or to describe the best relationship between Y and X.

What happens if we need to predict X from Y? If there is no meaningful regression of X on Y in this situation, it must be done by using the regression of Y on X in reverse[5]. The need for this arises principally in bioassay work, where concentrations of drugs or pollutants have to be estimated from the response of experimental animals. The problem of estimating confidence limits in these circumstances has been dealt with by authors such as Eisenhart (1939) and Winsor (1946).

[5]Recently Krutchkoff (1967) has claimed that the regression of X on Y is preferable in calibration situations, on the basis of Monte Carlo trials. However the work has been criticized, and Halperin (1970) concluded that Krutchkoff's results apply only in certain trivial cases. The argument may not be over, but meantime the "classical" procedure of Eisenhart (1939) and many subsequent authors seems the safest choice.

X Subject to Error, with Measurements Controlled

27. Berkson (1950) examined the status of regressions computed from data in which Y is variable without constraint, while X is variable but is "controlled." Quoting Berkson, "an 'uncontrolled' observation is made when, wishing to ascertain the value of some unknown quantity u_i, we measure it with an instrument... A 'controlled' observation is one made when, instead of wanting to know the value of some unknown u_i, we wish to bring the quantity to a value u_i." Berkson also defines a "modified controlled experiment" in which "the values of the independent variate are adjusted to begin with to specified values, as are controlled observations, but these values are not specified precisely..."

Berkson concluded that where the X variate is controlled, the ordinary predictive regression of Y on X (our expression 2) will also estimate the functional regression, or in his words the "true law," *even though the X measurement is subject to error.* In a 'modified controlled' situation "the line will be biassed but the bias can be reduced to a negligible amount by selecting values of X to cover a wide range." Table 3 compares the relation between true and observed values in the three types of experiment.

There was some controversy about Berkson's discovery (Kendall 1952, p. 97), but Lindley (1953) corroborated his results. Two models of an experiment with X controlled have been constructed along the lines of panel A of

Table 3. Relation between true and observed variates in experiments with controlled X, modified controlled X, and ordinary sampling. In each case the true relationship is Y = 2X and the absolute errors are the same.

Target X	Distance off target	Observed X	Error in X	True X	True Y	Error in Y	Observed Y
			A. Controlled observations				
4	0	4	+1	3	6	0	6
6	0	6	0	6	12	+2	14
8	0	8	−2	10	20	0	20
10	0	10	−1	11	22	+4	26
12	0	12	0	12	24	−2	22
			B. Modified controlled observations				
4	−1	3	+1	2	4	0	4
6	−1	5	0	5	10	+2	12
8	0	8	−2	10	20	0	20
10	0	10	−1	11	22	+4	26
12	+1	13	0	13	26	−2	24
			C. Ordinary sampling				
–	–	5	+1	4	8	0	8
–	–	6	0	6	12	+2	14
–	–	6	−2	8	16	0	16
–	–	9	−1	10	20	+4	24
–	–	12	0	12	24	−2	22

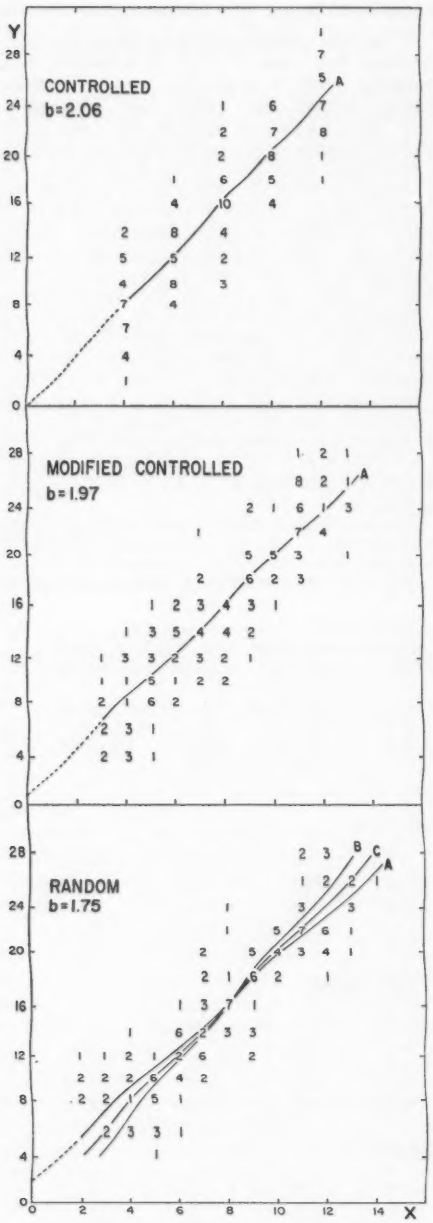

FIG. 6. Artificial frequency distributions of measurements made as follows:
Top: Berkson's controlled procedure
Middle: Modified controlled procedure
Bottom: Random sampling
In each case the true value of Y is twice the true value of X. See text for details.

Table 3 by making 30 "observations" of X at each of 5 predetermined values: 4, 6, 8, 10, and 12. These observations are liable to error distributed approximately normally,[6] the frequency distribution of the deviations being as follows:

Deviation	*Frequency*
0	6
+1 and −1	4 each
+2 and −2	1 each

One of these 16 deviations was selected randomly and added to an observed X, the result being the true value of X. To each true X there corresponds a true Y twice as large. Y measurements are subject to the same mean *relative* error as X, so to obtain observed Y a random selection was made from a series of deviations like the above but twice as large. Models of the same format and using the same series of random deviations were constructed for a "modified controlled" situation, in which the observed X could differ from the target X by 0, +1, or −1, with equal probability, as in Panel B of Table 3. Figure 6 shows the distribution of Y values in a controlled and a modified controlled situation, as compared with random sampling.

The statistics obtained from these models are shown in Table 4. For the controlled situations the values of b are close to 2, the true functional regression — thus confirming Berkson's theoretical analysis. The modified procedure shifts these estimates somewhat, the mean change being a decrease of 0.028, which is in the direction expected. In general the magnitude of such a shift depends on how accurately the target value is achieved, so it is evidently desirable to keep as close to the target as possible. Table 4 also contains the regression of X on Y and the GM regression, to show that these are not useful as descriptions of population characteristics in this situation.

Notice also that b will estimate the functional regression in the "controlled" situation *regardless of the relative size of the true errors in X and Y* (compare Section 29). For example, if the Y errors in the second controlled trial of Table 4 are doubled, it changes b only from 2.003 to 2.023, while a becomes −0.03; the correlation of course decreases substantially, to $r = 0.768$.

28. To obtain a functional regression from controlled observations it is important that the procedure be exactly as described by Berkson (1950) and illustrated above. *This procedure cannot be used with naturally-variable X distributions.* In Fig. 4, for example, 5 measurements of Y were used at each X, but the resulting b

[6]It is not essential that the error be normally distributed; if the deviations are all given equal probability the result is very similar.

was an estimate of the predictive regression only, and not the functional. The same applies if the number of X categories in Fig. 4 is reduced to 4 or 5, even though this produces a graph that superficially resembles the top panel of Fig. 6.

Random Sampling

29. In most situations where the error in X arises solely in the measuring process, observations will be either controlled or modified controlled, in Berkson's sense. That is, experimenters usually arrange to make observations at predetermined regular intervals of time, temperature, size, velocity, etc., rather than sampling at random from the whole spectrum of possible values. However, there are also situations in which an investigator has to take what observations he can get, without having any target figures. In that event expression (2) for b estimates only the predictive regression of Y on X, and is not equivalent to the functional regression.

When both Y and X are subject to measurement error only (no natural variability) it is possible, in principle at least, to obtain the least-squares estimate of the functional regression by getting supplementary information. What is needed is an estimate of the ratio, λ, of the true variance of Y at a given (true) value of X (called the "point" variance) to the true variance of X at given Y (Lindley 1947). Given the functional relationship:

$$Y = g + hX \tag{24}$$

h is estimated by the positive root of:

$$h^2\sum xy + h(\lambda\sum x^2 - \sum y^2) - \lambda\sum xy = 0 \tag{25}$$

which is:

$$h = \frac{\sum y^2 - \lambda\sum x^2 + [(\lambda\sum x^2 - \sum y^2)^2 + 4\lambda(\sum xy)^2]^{\frac{1}{2}}}{2\sum xy} \tag{26}$$

Notice that when $\lambda = 0$ (no error in Y) expression (26) reduces to $\sum y^2/\sum xy$, the reciprocal of the predictive regression of X on Y; while as the error in X approaches zero, λ becomes indefinitely large and expression (26) approaches $\sum xy/\sum x^2$, the predictive regression of Y on X — the situation discussed in Section 26 above. *Thus the two predictive regression estimates set limits to the best estimate of the functional regression.*

Table 5 illustrates the use of expression (26) by means of 5 random-sampling models. In columns 4–6 the construction was as in Panel C of Table 3. In columns 3, 4, and 7 the same random selections were used for X and (multiplied by 1, 2, or 4) also for Y (but in a different order, of course); hence the point variances of Y are, respectively 1, 4, and 16 times the point variance of X. In columns 5 and 6 different random series were used for X and Y, but the ratio λ is still very close to 4. In each case the population value of the functional regression is 2, and the 5 estimates of it agree excellently (line h).

30. In practice, the ratio λ required in (26) is rarely available when all the variability results from errors of measurement. It requires special observations on Y when X is accurately known, and on X when Y is accurately known; and if such accuracy in measuring X is attainable, it is usually much easier to employ it in routine measurements so that we have the simpler situation of Section 26. What can *not* be done, unfortunately, is to estimate the ratio of the point variances of Y and X in each individual body of data from the observed variances of the data points from the two predictive regression lines. The reason is that the ratio of the latter is *always* proportional to the ratio of the total variances, because of the identity:

$$\frac{s^2_{yx}}{s^2_{xy}} = \frac{\sum y^2 - (\sum xy)^2/\sum x^2}{\sum x^2 - (\sum xy)^2/\sum y^2} \equiv \frac{\sum y^2}{\sum x^2} \tag{27}$$

31. In the absence of any objective estimate of the value of λ in expression (26), three courses seem possible. The first is to make one or more guesses at the value of λ on the basis of any approximately quantifiable indications or hypotheses that can be gleaned from the

Table 4. Statistics computed from models of "controlled" (C) and "modified controlled" (MC) observations, constructed as in Table 3. The observational errors in the two series of each trial are the same; they differ only in that the X observations can be "off target" for MC. For comparable series of random observations, see Table 5, columns 5 and 6.

		First trial		Second trial	
		C	MC	C	MC
Correlation	r	0.891	0.887	0.883	0.888
Regression of Y on X	b	2.057	1.974	2.003	2.029
Standard error of b	s_b	0.0854	0.0841	0.0872	0.0858
Intercept	a	−0.15	+0.38	+0.08	−0.22
Regression of X on Y	d	(0.386)	(0.398)	(0.389)	(0.388)
GM regression	v	(2.310)	(2.227)	(2.270)	(2.287)

TABLE 5. Regressions computed from random-sample situations, using different ratios of the point variability of Y to the point variability of X (= λ).

1	2	3	4	5	6	7
		Value of λ				
		1	4	4	4	16
Correlation	r	0.919	0.874	0.877	0.893	0.744
Predictive regression of Y on X	b	1.729	1.714	1.749	1.758	1.721
Predictive regression of X on Y	d	0.489	0.446	0.440	0.454	0.321
Least squares estimate of functional regression	h	1.974	1.956	1.993	1.964	1.964
GM regression of Y on X	v	1.881	1.962	1.994	1.967	2.314
Standard error of b and v	s_b, s_v	0.0604	0.0779	0.0841	0.0722	0.1263

nature of the material.[7] If no educated guess is possible, there is a choice between two conventional values: either λ can be put equal to unity, or it can be made equal to the ratio of the total variances of the two sets of observations. The latter condition is:

$$\lambda = \sum y^2 / \sum x^2 \qquad (28)$$

At first glance $\lambda = 1$ might seem the less arbitrary choice. However when $\lambda = 1$ expression (26) reduces to (19), which is the major axis of the correlation ellipse, and the position of this line relative to the data points will then depend partly on the choice of the units in which X and Y are measured. For example, weight measured in kilograms gives the line a very different position among the points than does weight in grams (Section 15). Using condition (28), however, (26) reduces to the GM regression line of expression (16), whose position relative to the data points is independent of the measurement units employed.

Thus we come to the conclusion that the GM regression is the best available estimate of the functional relationship (based on the least-squares principle) for the situation where all the variability of both variates is due to measurement error *and* there is no supplementary information concerning the relative point errors in X and Y. This usage was apparently first recommended by Dent (1935), and Sprent (1969, Chapter 3) has a good modern discussion of the pros and cons. Although in this situation the GM regression is not a "consistent" estimate of the functional relationship, in the long run of experience it will provide estimates without any *mean* bias, even though each individual estimate can be expected to have some bias in one direction or the other.

Some idea of the actual bias can be had from the models of Table 5. Here $\sum y^2 / \sum x^2$ is approximately 4 in all cases, so that when $\lambda = 4$ the GM regression is very close to the true functional regression. When $\lambda = 1$, instead of 4, the GM regression moves about $\frac{1}{3}$-way toward the predictive regression of Y on X; similarly when $\lambda = 16$ the GM regression moves away from the predictive regression of Y on X by an amount about equal to that distance.

32. If the GM regression is used because λ is unknown it is necessary to be cautious about the confidence limits of Section 9 (or any others that may be developed). Strictly speaking they are not applicable at all. However for *all* values of λ the best estimate of the functional regression must lie between the estimates b and $1/d$, whereas its true value can lie outside these limits; frequently the 95% confidence limits for an estimate of v greatly exceed the interval between b and $1/d$. Unless correlation is very weak, therefore, confidence limits computed as in Section 9 will give a fairly good idea of the probable *range* of random variability in v in this situation, even though these limits will in general not be precisely positioned because λ is in fact not exactly equal to $\sum y^2 / \sum x^2$.

D. Combinations of Inherent Variability and Measurement Error

33. In practice there seem to be few situations in biology where both Y and X are subject to measurement error alone, so that opportunities to use expression (26)

[7] Jolicoeur and Heusner (1971) use this approach. In their table 5 they list a series of values for the slope of the line (relating logarithm of basal metabolism to logarithm of body weight in rats) that lie between $1/d$ and b, i.e. from $\lambda = 0$ to $\lambda = \infty$; and they speculate that the true value of λ might be between 2/3 and 3/2, corresponding to slopes (exponents) of 0.724 and 0.681. However, since variability in their data is largely natural, the GM regression is a unique best estimate of the exponent they are seeking; its slope is 0.737, corresponding to $\lambda = 0.544$. This tends to support Kleiber's mean exponent of 0.75 for mammals generally, rather than the surface-law value of 0.67.

together with supplementary information concerning λ are quite rare. I personally have not encountered any. On the other hand, mixtures of natural and observational variability are common, but in that event there seems no obvious way to estimate an overall λ, even in principle. That is, it may be possible to assess the observational component of variability for X and Y separately, but not the natural component. In Section 8 it was shown that the GM regression is the appropriate estimate of the functional relationship for purely natural variability. Hence the larger the natural component of variability in a mixed situation, the better the GM regression becomes as an estimate of the true functional regression. With biological materials very frequently 80% or more of the variability is natural, and for practical purposes this can be treated as 100%. Even if the measurement component of variability is considerably greater than 20%, this will prejudice the use of the GM regression only if the point measurement variance of X, relative to the total variance of X, is substantially different from the same ratio for Y (or in other words, if the condition of expression (28) is seriously violated). Hence in a great majority of biological situations the GM regression can be used without reservation as a reasonably consistent estimate of the functional slope.

If there is doubt, measurement variances can be determined directly if concrete objects are being studied, simply by making a number of replicate observations of Y and of X on the same object (or pair of objects), and computing the two variances and their ratio λ. In the example of the weight: length relationship of Section 47, this would mean repeated measurements of the length and weight of a single fish, using the same observers, technique, and equipment as for the sample data. An alternative procedure, which is preferable if some degree of non-homogeneity of variance may exist, is to make duplicate measurements of Y and of X routinely on all the objects studied. If we call these Y_1, Y_2 and X_1, X_2, it is easy to show that the observational variance of Y is $\sum(Y_1-Y_2)^2/2N$ while that of X is $\sum(X_1-X_2)^2/2N$. A comparison of such observational variances with the corresponding total variances from the regression lines, s^2_{yx} and s^2_{xy}, will show how large a fraction of the latter is due to errors in measurement. Similarly the ratio (λ) of the observational variances obtained by either of the above methods can be compared with $\sum y^2/\sum x^2$ to show whether the condition of expression (28) is approximately satisfied. Only if measurement variance is relatively large *and* (28) is poorly satisfied is it necessary to consider the alternative method of estimating the functional regression described in Section 34.

Functional Regressions from Arithmetic Averages (AM Regressions)

34. By dividing a distribution into segments and computing the arithmetic mean of X and of Y for each, estimates of the functional regression can be calculated for which the claim has been made that they are consistent and independent of the ratio λ. There have been two proposals.

Wald's method — The pairs of variates are arranged in order of increasing values of X and are then divided into two equal groups, 1 and 2, the central member of an odd-numbered series being omitted (Wald 1940). For each group the sum of the X values and of the Y values is calculated. In the expression:

$$Y = j + kX \tag{29}$$

the slope is found from:

$$k = \frac{\sum Y_2 - \sum Y_1}{\sum X_2 - \sum X_1} = \frac{\overline{Y}_2 - \overline{Y}_1}{\overline{X}_2 - \overline{X}_1} \tag{30}$$

Graphically, this means joining the mean points of the two halves of the distribution, as illustrated in Fig. 5 above. The value of j is estimated by substituting k for b in expression (4). Wald also demonstrates a method of computing confidence limits for k.

Nair–Bartlett method — This resembles Wald's method, but the material is divided into three groups (Nair and Shrivastava 1942; Nair and Banerjee 1942; Bartlett 1949). Both Nair and Bartlett claim that this is somewhat more efficient than using only two groups, but this apparently applies only to the large-sample situation. If N is small it seems better to use only one division (Wald's method) and so have less sampling fluctuation. In the Nair–Bartlett method the outer two groups (1 and 3) are made equal and the middle one is as close to N/3 as possible. For the functional relation:

$$Y = m + nX \tag{31}$$

the slope is estimated as:

$$n = \frac{\sum Y_3 - \sum Y_1}{\sum X_3 - \sum X_1} = \frac{\overline{Y}_3 - \overline{Y}_1}{\overline{X}_3 - \overline{X}_1} \tag{32}$$

Again the line (31) is located by passing it through ($\overline{X}$, $\overline{Y}$), as shown by expression (3)[8].

[8]My first acquaintance with measuring slope by this method came indirectly from the Faculty of Forestry, University of Toronto, where it was in use during the 1920s, though I have not yet discovered who originated it. However the foresters located their line by measuring off $\frac{2}{3}$ of the distance from the point ($\overline{X}_2$, $\overline{Y}_2$) to the line joining ($\overline{X}_3$, $\overline{Y}_3$) and ($\overline{X}_1$, $\overline{Y}_1$), and passing the regression line through that point. As far as I know they did not distinguish between a predictive and a functional regression. Nair and colleagues have the first published reference known to me that describes this method of determining slope, which they identified as the functional regression, but they located the regression line simply by joining ($\overline{X}_3$, $\overline{Y}_3$) and ($\overline{X}_1$, $\overline{Y}_1$). Bartlett once again developed this method of obtaining the slope, and he was the first to combine it with the normal procedure of passing the regression line through the point representing means of all variates ($\overline{X}$, $\overline{Y}$).

35. Confidence limits for n and m are derived by Bartlett (1949), and the steps in their computation are presented systematically by Simpson et al. (1960). The sums of squares and cross-products must be computed for each of the 3 groups of variates *using deviations from the mean of each group separately*. The two outer groups of course contain the same number of variates, K. The sums of squares and products for each of the three groups are then summed to give totals which we may designate by the symbols $\sum' x^2$, $\sum' y^2$ and $\sum' xy$ respectively, and the t-value for N–3 df at the desired level of significance is looked up in a table of Student's distribution. The expression for estimating confidence limits for m is:

$$2t^2\mathrm{K}(\textstyle\sum' y^2 - 2\beta\sum' xy + \beta^2\sum' x^2)$$
$$= (\mathrm{N} - 3)\,(\textstyle\sum \mathrm{X}_3 - \sum \mathrm{X}_1)^2\,(m - \beta)^2 \qquad (33)$$

This is a quadratic equation in β, and its two roots are the confidence limits for m at the chosen level of significance.

36. It is easy to show that the AM regressions, as originally proposed and as used to date, do not in fact provide unbiased estimates of the functional regression except in one rather trivial situation. This is when the same sequence of pairs of variates is obtained (at the places where a division has to be made) by arranging them in the order of Y as by arranging them in the order of X. However in biological practice it is quite unusual to find that the two orders divide the distribution into exactly the same two (Wald) or three (Nair) segments: this happens only when the correlation is close (usually $r > |0.9|$) and the variates are few, and not always even then.

In dividing the variates by the order of X, or of Y, we create situations analogous to the "Type B" situations discussed in Section 17. For example, if the distribution of Fig. 1 is divided into two halves on the basis of the brothers' heights and Wald's computation is made, the functional slope estimate obtained is only about half as great as when the division is made by the order of sisters' heights. (There is of course a technical problem in dividing such a series because there are many Y observations at each X, and vice versa; within the X category in which a division falls it is necessary to assign the Y values to each segment by means of a random selection process.) To make the estimate symmetrical with respect to X and Y, the variates can be divided transversely to the trend (in which event the problem of dividing a long series may become even more bothersome). Another possible procedure is to divide the series twice, once by the order of X and once by the order of Y, then add the numerators and denominators of the two expressions for k (expression 30) in order to obtain a symmetrical quotient that can be taken as the estimate of the functional regression (or similarly for expression 32).

37. In addition to all this, the observational error component in the variability of X or Y may "misplace" two or more pairs of variates. When this occurs it introduces additional uncertainty bias into the slope estimate, and hence confidence limits computed by Wald's or Bartlett's methods are too narrow (Wald 1940; Madansky 1959).

Finally, simply as a matter of observation, all the symmetrical AM estimates of the functional regression that I have calculated have been somewhat smaller (ignoring the sign) than the GM regression computed from the same material. This is true both for estimates made from data divided transversely to the trend and for those made by combining the order of X and order of Y. In most of these examples, amounting to 15 or so, the GM regression was known to be theoretically sound because of the nature of the material. Hence there is a suggestion that there is a small consistent bias inherent in the AM procedure even when it is made symmetrical. Additional theoretical or practical investigation is needed to confirm or reject this possibility.

38. It appears then that the AM methods present both theoretical and technical difficulties sufficient to make one wish to avoid them if at all possible. To illustrate this with a short series, Table 6 and Fig. 7 show a selection of 11 pairs of variates made by Snedecor (1946, p. 138) from the distribution of Fig. 1. Since nearly all the variability in this series is natural, the GM regression is the appropriate estimate of the functional regression; it is $v = 0.944$. This is of the magnitude we would expect, not only because we happen to know v for the parent distribution (Section 11) but also from the nature of the material: since the mean heights of the women and men in this Table are in the ratio of 64:69, or 0.927, it is likely that the functional relation will be approximately the same.

Considering now the AM regressions in Table 6, we find that they have widely different values depending on whether the variates are arranged in order of brothers' heights (a) or sisters' heights (b). This illustrates the inappropriateness of the AM methods as originally proposed: two of the values even lie a little outside of the possible range for a best estimate, set by b and $1/d$. The order suggested by the trend (c), and the combination of (a) and (b), give more reasonable functional estimates, but they are all less than the GM regression. Confidence limits by Bartlett's method are shown for the trend order; they are relatively wider than the approximate limits for the GM regression, and this is true in general.

Clearly the AM methods give a poor result in this example, which is not at all atypical among those I have encountered (see also Section 55). It is perhaps fortunate that these methods have not yet been employed very widely, for unless used symmetrically their estimates are likely to be closer to the corresponding predictive regression than to the true functional regression (see Section 53). Larger numbers of variates, or less variable data, will of course reduce the difference between the true functional regression and the estimates made by *symmetrical* AM methods. However, as things stand at present, it seems impossible to be sure that an AM regression will ever give a sounder estimate than the GM regression.

Regressions that Pass Through a Fixed Point

39. Consider the situation where $u = 0$ in our expression (15), that is, fitting a functional line that must pass through $X = Y = 0$. Thus (15) is modified to become:

$$Y = pX \tag{35}$$

For the case where both Y and X are normally distributed, Creasy (1956, Section 4) shows that the maximum likelihood estimate of the functional slope p is:

$$p = \frac{\sum Y}{\sum X} \tag{36}$$

This line passes through the point $(\overline{X}, \overline{Y})$, so it can be drawn graphically by joining the means of all observations to the origin.[9]

[9]Expression (36) is also the best *predictive* regression if the variance of Y about the line is proportional to X, instead of the assumption (used throughout this paper) that it is independent of X. For the latter situation the best estimate of the predictive regression is $\sum XY/\sum X^2$ (Deming 1943, Section 14). However, for open-ended distributions the functional regression (36) will be the best one available for prediction.

Expression (36) does not require any information concerning the value of λ (Section 29), hence it can be used whether the variability in X is natural or from errors in measurement, or a combination of these. How (36) is affected by non-normality of the distributions has not been studied, but by analogy it seems likely that it will be useful for any series of data that has been obtained symmetrically with respect to the trend.

40. The variance of the points from the functional regression line (35) is given by Creasy as:

$$\frac{\sum y^2 - p\sum xy}{N - 1} \tag{37}$$

The problem of computing confidence limits for p has perhaps not been completely solved. Creasy recommends a formula (her expression 9) as "the best available", but it requires considerable computation and might be little better than simply dividing (37) by $\sum x^2$ and treating the square root of the result as an ordinary standard error with $N - 1$ df. The latter procedure can at any rate be used for orientation.

41. If a line must pass through a fixed point other than the origin, the appropriate coordinate of that point

TABLE 6. A: Adult heights of 11 pairs of brothers and sisters in inches, arranged in the order of the trend (Fig. 7), and basic statistics. B: Regression computations.

A. Data

Brother, X	65	66	67	66	68	71	70	70	73	72	71
Sister, Y	59	62	63	65	64	62	65	65	64	66	69

$\overline{X} = 69, \overline{Y} = 64, \sum x^2 = 74, \sum y^2 = 66, \sum xy = 39, r = 0.558$

B. Slopes and standard errors of the regressions

	Slope	St. error	95% limits	
Predictive regression of Y on X (= b)	0.527	0.236	−0.007	+1.161
Predictive regression of X on Y (= d)	0.591	0.265	−0.009	+1.191
Reciprocal of the above (= $1/d$)	1.692			
GM functional regression (= v)	0.944	0.236	+0.410	+1.478
Wald's functional regression (= k)				
(a) order of X	0.520			
(b) order of Y	1.667			
(c) order of the trend	0.667			
(d) combination of (a) and (b)	0.892			
Nair-Bartlett functional regression (= n)				
(a) order of X	0.522			
(b) order of Y	1.357			
(c) order of the trend	0.682	–	+0.039	+1.559
(d) combination of (a) and (b)	0.838			

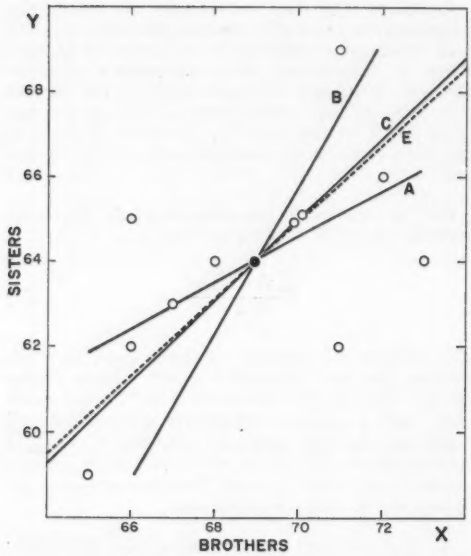

FIG. 7. Graph of the brother–sister data of Table 6. Open circles: observed heights; solid circle: mean. Lines A and B are the predictive regressions; C is the geometric mean regression; E is the major axis of the correlation ellipse.

should be subtracted from each variate before applying the expressions above.

Examples of Regression Situations

42. In deciding what regression to use and how to proceed, an investigator should ask himself a series of questions such as the following:

1. Are the data subject to natural variability, or measurement error, or both?
2. Is one of the variates obviously primary or casual so that it would conventionally be the "X" variate?
3. Is the X variate free of error, for practical purposes?
4. If only measurement variability is involved in X, have the measurements been made randomly, or are they controlled (in Berkson's sense)?
5. If the variability is wholly or mainly natural, are the distributions of the two variates symmetrical and reasonably close to normal?
6. Should symmetrical stratified sampling be used to avoid having an unmanageable abundance of intermediate values and so get a more even distribution of information?
7. Does a (non-normal) bivariate distribution have fixed limits or is it "open-ended"? In either event, has it been sampled symmetrically with respect to the trend?
8. For such a distribution, is the variance of Y independent of the value of X? If not, can a transformation of Y be used?
9. Is there any fixed point through which the line *must* pass?
10. Is the regression needed to make predictions, or to describe a relationship, or both?
11. If both, must the same relationship be used for prediction and for description, or can different lines be used for these two purposes?

Some of these questions will be illustrated in situations of common occurrence in fishery work.

Conversions Between Different Length Measurements

43. A common use of regression lines is to convert between the different types of lengths used in measuring fish. This is a predictive situation and would appear to require a predictive regression. However, the population of measurements to be envisaged here is of the "open-ended" type defined in Section 21; furthermore, usually only a part of the length distribution of the fish is represented in the sample. In general, also, observations will not become unavailable at a given length (of either sort), but will peter out transversely to the trend, so that the sample is symmetrical with respect to the trend line. Accordingly the functional regression is the best one to use for prediction; and its estimate has no systematic bias related to the range of lengths represented in the sample. The predictive regressions, by contrast, tend to increase as the range of lengths available increases.

In addition, predictive regressions can create a problem when there is need to convert both ways: say standard to fork length, and fork to standard. Suppose we do use two predictive regression lines, each one for making the conversion in the appropriate direction. It can happen that we need to convert a given standard length to fork length (Q to P in Fig. 8), then convert it back again later (P to R), perhaps as part of an average that includes fish measured in fork length. Using the two regressions, we find that during the process the original standard length has become larger if it was less than the mean (it moves from Q to R in Fig. 8), or smaller if it was larger than the mean.

For an estimate of the functional regression in this situation the geometric mean estimate of expression (16) is suitable. This is partly because the variances of the different methods of measuring length are approximately the same and the range of lengths involved is not very different, hence (28) applies; also, one component of the variability (probably usually the larger one) is inherent in the material itself.

Summarizing, for routine length conversions the GM regression is to be recommended for converting in either direction or in both directions.

Calculation of Fish Lengths From Scale Measurements

44. The relation between a fish's length and the radius of a particular scale may be linear, simply-curved,

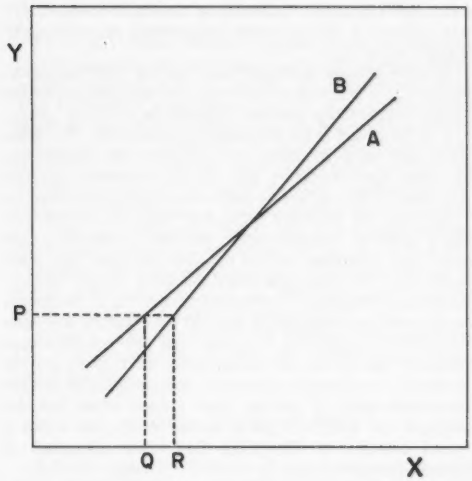

FIG. 8. Illustration of 2-way conversion between standard length (X) and fork length (Y). A is the regression of Y on X; B is the regression of X on Y (See the text.)

or S-shaped. For the first of these situations a straight line can be fitted directly, and for the second a straight-line transformation can usually be found (a double logarithmic transformation is sometimes used even when the line appears straight, in order to stabilize variance). The S-shaped situation is less tractable and a freehand line may then be the best procedure available. In all cases the problem is to calculate the length of a fish at earlier stages of its life from the "checks" or "annuli" on the scale.

Since the line will be used to compute fish lengths from scale radii, it would appear that the correct regression to use should be the predictive regression of length on radius. In fact, however, this line tends to underestimate lengths that are greater than the mean of the sample used to establish the relationship, and to overestimate those less than that mean. The reason is simply that the population of measurements to be envisaged is again of the uniform or open-ended type described in Section 21; hence the functional rather than either predictive regression should be used for prediction. Since measurement errors of both fish and scales are minor relative to the natural variability, the GM regression is appropriate.

An additional consideration that makes the predictive regression unsuitable is the fact that the population of scale measurements to be converted to lengths is never the same as the population of scale measurements from which the regression was computed, even when the same fish population is involved, thus violating Lindley's dictum quoted in Section 20. The reason is that in the process of back-calculation all the scales used will have a first annulus, most or all will have a second, and so on — decreasing to very few scales for the oldest age present. To establish the relationship, however, it usually happens that there are fewer body/scale measurements in the region of ages 1, 2, etc., than at a number of older ages; in fact sometimes there are no young fish at all and the line obtained must be extrapolated back as far as the shortest first-year annulus observed. Such an extrapolation of a predictive regression for a non-normal distribution is unavoidably unrepresentative, whereas extrapolation of a functional regression need not be so.

To improve the representativeness of the body/scale relationship some have averaged the fish lengths at successive short intervals of scale radius, and have given each such interval equal weight when fitting a predictive line. Under some circumstances this may improve the fit of the *predictive* line (for example, if we are really computing the best linear approximation to a relationship that is in fact not quite linear), but it destroys any possibility of using the more appropriate functional regression (Section 24).

45. Insofar as back-calculated lengths are progressively too large at younger ages (and this is true of many published series) it means that growth during age 0 is overestimated, during age 1 it is underestimated, during age 2 it is also underestimated but less so, and so on up to the middle group of ages in the sample used to establish the regression line[10]. This effect of course is quite apart from the possibility that the scales being back-calculated may tend to be from fish larger than the average for their age-group. This latter is an ever-present danger that often produces a similar bias (Ricker 1969).

46. In a minority of back-calculation situations the appropriate model is direct proportionality between fish length and scale radius, in which event expression (36) should be used to establish the position of the regression line. The above discussion of how data are to be collected, or selected, applies here also.

WEIGHT:LENGTH RELATION

47. A frequent use of regression lines in fishery work is for estimating the weight: length relation. Usually the investigator is interested in establishing the functional form of the relationship, the expression describing the relation between weight and length *in the fish population*. The relation assumed to be appropriate is of the form:

$$\log w = \log\alpha + \beta(\log l) \qquad (38)$$

or in antilogged form:

$$w = \alpha l^{\beta} \qquad (39)$$

Much the greater part of the variability in a series of weight–length observations will be inherent in the material, rather than in the process of measuring length or

[10]Actually the regression of scale radius on fish length has been used for back-calculations by some authors (including, at one time, the present writer), in which event the above biases are reversed.

weight, so the geometric mean regression of expression (16) is an appropriate estimate of the functional relationship.

48. If conversions of length to weight are the sole concern, the considerations that apply are discussed in Section 21 above; they are in fact the same as when converting between lengths (Section 43). Hence the GM line should be used for estimating weight from length, or length from weight.

In the past the predictive regression of log weight on log length has nearly always been used to estimate the exponent β in this relationship. Because of this, most of the exponents in the literature have a bias in the direction of being too small. These can be converted to functional estimates if the standard error or the correlation coefficient is given, by using the appropriate portion of expression (17). To establish the position of the Y-axis intercept it is necessary to know either $\overline{X}$ or $\overline{Y}$ as well, or preferably both.

49. Suppose that we wish to compare two populations as regards weight:length relation, one population consisting of much larger fish than the other; and suppose further that the two populations are in fact identical in respect to weight:length relation. The GM regression provides an estimate of the slope of the regression, in each case, that has no systematic bias related to the range of lengths involved in each of the two series being compared, and the estimate of the Y-axis intercept is also unbiased.

If the predictive regression of log weight on log length were to be used, the slopes obtained would reflect, in part, the range of sizes available (greater slope for longer range), and the intercept would depend partly on the absolute size of the fish in each population (compare lines A and B in Fig. 5).

50. As another illustration, consider the situation where a weight:length relationship has been computed for all the fish in a sample, and also for each age-group individually[11], using the predictive regression of expression (1) in each case. It is commonly found that the within-age slopes are considerably less than the overall slope. Much of this difference, however, can result from the shorter length series available for the individual ages: this automatically tends to make their b values smaller than that for the complete series (Fig. 5). To test whether there is any real difference, functional regressions should be used.

Morphometric Analysis

51. The weight:length relation above is one of a large class of morphometric relations that are linear in a double logarithmic presentation. Most of these are relations between two length measurements. In these cases the *functional* regression coefficient is usually close to unity; if it is exactly 1, the two parts are growing proportionally isometrically.

In 1948 Teissier recommended that the GM regression be used as the principal (but not the exclusive) estimate of the relationship between logarithms of body parts; in fact he called it *the* allometric relationship. We differ from him only in seeing no occasion for exceptions, provided measurements are made accurately enough that most of the variability will be inherent in the material.

In spite of Teissier, predictive regressions are still being used in morphometric analysis. Commonly the body part (diameter of the eye, for example) has been made the Y-variate and standard body length the X-variate, and the predictive regression of Y on X calculated. Such predictive regressions tend to have values less than 1, which is interpreted to mean that the eye becomes relatively smaller as the fish grows — that is, its growth is allometric rather than isometric. But since the predictive regression tends to be less than 1 even when (on the average) eye and body grow isometrically, this is not a sound conclusion. The GM functional regression is what is needed.

Estimating the Catchability of Fish

52. The central feature of the Beverton-Holt method of estimating fish population parameters is a regression of total instantaneous mortality rate, Z, on fishing effort, f, over a series of years. The slope of the line obtained is an estimate of the *catchability*, q, of the fish (the fraction of the stock taken by 1 unit of fishing effort). From this the instantaneous rate of fishing for any level of fishing effort can be estimated from $F = qf$. Also, the mean instantaneous *natural mortality rate*, M, in the stock is the value of Z when $f = 0$, or in other words the ordinate intercept of the regression line.

The above applies without reservations to the situation where the correlation between f and Z is perfect. In practice the correlation is far from perfect, so the question arises, which regression is appropriate? Evidently we need a "reversible" value of q to use in $F = qf$, and q must also be consistent with some reasonable value of M. To date the predictive regression of Z on f has usually been used, but insofar as there is error in f, all the estimates so obtained have a bias: q in the direction of being too small and M in the direction of being too large.

Paloheimo (1961) drew attention to this problem, and suggested substituting for the Beverton-Holt model an approximate linear model that has a direct algebraic solution and less variability in the estimates obtained. Also, in the artificial examples he examined, the linear model showed little evidence of bias in the mean estimates of q and M that were obtained.

Actually the standard errors of estimates of M made by either the Beverton–Holt or the Paloheimo procedure are frequently so large as to make the estimate useless. In that event it is better to look to a different source for an estimate of M — usually the natural age structure of the population — and use it to improve the estimate of q. The procedure is to fit a functional line to pass through the point ($f = 0$, $Z = M$), as described in Section 41.

[11]Qualitatively these are different concepts: the one mainly reflects change with age, the other does not. There is no reason that they should be identical, but it is of interest to compare them.

Schaefer's Method of Population Analysis

53. Apart from Teissier's applications to morphometry, the first use of a functional regression in fishery work apparently was by the late M. B. Schaefer (1957). The method of population analysis used in that paper involves the relation between an estimate of equilibrium catch per unit of effort (Y_E/f) and an estimate of fishing effort (f). For a yellowfin tuna population, with 20 pairs of points available he used the Nair-Bartlett estimate (expression 32 above), arranging the variates in the order of f. The slope estimate was –0.164, only slightly different from the predictive regression of Y_E on f (–0.157). However if the variates are arranged in the order of the trend, this slope becomes –0.195; the GM functional regression gives a similar value, –0.211.

Using a slope of –0.195 and corresponding intercept, the equation relating equilibrium yield (Y_E) to fishing effort becomes:

$$Y_E = 11710f - 0.195f^2 \qquad (41)$$

Schaefer used the functional regression only for purpose of description, and went back to the predictive regression for the purpose of estimating the equilibrium catch to be expected from any given number of days of fishing effort. However, since the range of efforts available was very incomplete at both ends, the functional regression is also best for predicting. Differentiating (41) and equating to zero, the maximum sustained yield is estimated to be taken with 30,100 days of fishing effort, and to be 176 million pounds per year. This is somewhat less than Schaefer's estimated maximum: 193 million pounds taken by 34,200 days of fishing. There are of course more recent data concerning this relation.

Routine Metabolic Rate of Fishes

54. Winberg (1956) summarized measurements of routine metabolic rate, that is, the oxygen consumption of quiescent fishes (Q, in ml per gram of fish per hour at 20° C) of different weights (w, in grams). He computed the predictive regression (b) of log Q on log w for various groups and for the total. Using our symbols, the data are summarized in Table 7. Winberg uses the value of b to describe the relation between routine metabolic rate and body weight. However a functional regression is needed to describe a natural relationship of this sort, and the GM regression, v, is the most appropriate estimate of it because the variability in the data is likely to be mainly natural. And because the data series are typically non-normal and open-ended, a functional regression should also be used for prediction.

The net result of using v instead of b is to make metabolic rate somewhat less dependent on body weight than has been supposed; in other words, it is farther from the surface-law value of 0.667. Winberg uses a general average value of 0.80 for this exponent in his metabolic computations. The present interpretation of the data makes 0.85 a more appropriate figure. The unweighted

Table 7. Summary of Winberg's (1956) review of determinations of routine metabolic rate (Q) of fishes in relation to weight (w). Column 4 is the predictive regression of log Q on log w, and column 7 is the GM regression. Rows 12 and 16–19 are from Winberg's table 18, the value of v being the ratio of the standard deviations shown in that table. Other rows have been calculated from the data in Winberg's primary tables.

1 Kind of fish	2 Winberg's table	3 N	4 b	5 $s_b = s_y$	6 r	7 v
1. Goldfish (*Carassius auratus*)	3	23	0.807	0.054	0.952	0.848
2. Carp (*Cyprinus carpio*)	4	35	0.834	0.021	0.989	0.843
3. Tench (*Tinca tinca*)	7	17	0.794	0.041	0.978	0.812
4. Other Cypriniformes	8	48	0.848	0.030	0.972	0.872
5. Sturgeons (Acipenseridae)	5	33	0.817	0.012	0.996	0.820
6. Salmonoids (Salmonidae, Coregonidae, Thymallidae)	6	30	0.778	0.030	0.978	0.795
7. Cyprinodontidae	13	25	0.692	0.043	0.954	0.726
8. Pike (*Esox lucius*)	12	8	0.879	0.074	0.973	0.894
9. Eels (*Anguilla*)	10	19	0.833	0.034	0.984	0.846
10. Percoids (Percidae, etc.)	11	21	0.833	0.110	0.855	0.974
11. Lampreys (Cyclostomata)	9	5	0.849	0.139	0.939	0.904
12. All freshwater fishes	18	266	0.81	0.014	0.969	0.83
13. Marine fishes, mainly teleosts	14	51	0.857	0.032	0.966	0.887
14. Miscellaneous marine teleosts	15	37	0.812	0.044	0.949	0.856
15. Sharks (Selachii)	16	10	0.881	0.044	0.988	0.892
16. Young marine fishes (Zeuthen)	18	25	0.71	0.070	0.907	0.78
17. All marine fishes	18	123	0.79	0.014	0.969	0.81
18. All fish	18	389	0.78	0.0096	0.974	0.82
19. All fish except row 16	18	364	0.81	0.0105	0.971	0.84

mean of v for the 11 freshwater groups is 0.849. The data for saltwater fish are less numerous and less satisfactory: Winberg has reservations about the early work at Concarneau (row 13 in Table 7) and particularly about the work on young fish by Zeuthen (row 16), while the sharks are represented by only a small sample. However the unweighted mean of v for all 4 groups is 0.854, practically the same as that for the most representative sample (row 14).

Insofar as different species or groups may have different values for slopes and intercepts, it is somewhat better to use means of this sort rather than averages obtained from heterogeneous combined assemblages such as rows 12, 17, 18, and 19. However Winberg's overall figure (excluding Zeuthen) is recomputed in row 19 as 0.84, very close to the working mean value of 0.85 suggested here.

Relation of Fecundity to Body Weight in Fish

55. Sokal and Rohlf (1969, p. 483) tabulate data for 11 pairs of observations on the number of eggs in thousands (F) and body weight in hundreds of grams (w) of the cabezone, a cottid fish. From these the following statistics can be computed:

Correlation coefficient	0.880
Predictive regression of F on w	1.87
GM regression of F on w	2.12
Nair-Bartlett AM regressions	
Based on the order of w	1.80
Combined orders of F and w	1.99
Order of the trend	2.04

For an estimate of the functional regression Sokal and Rohlf recommend using the AM value of 1.80, based on

Table 8. Summary of regressions appropriate to different situations. See the text for definitions of symbols.

	Section references	For prediction of: Y from X	For prediction of: X from Y	Functional relationship
A. *Both X and Y subject to natural variability only; parental population bivariate normal*				
(a) Random sample	1–12	b	d	v
(b) Sample artificially truncated at a given value of X	17	b	–	–
(c) Sample artificially truncated at a given value of Y	17	–	d	–
(d) Sample artificially truncated transversely to the trend	17	–	–	v
(e) Y variates of the sample artificially limited at each X	18	b	–	–
(f) X variates of the sample artificially limited at each Y	18	–	d	–
(g) Equal combination of (e) and (f)	19	–	–	v
B. *Both X and Y subject to natural variability only; parental population "open-ended"*				
(a) Sample random or non-random, but symmetrical with respect to the functional axis	21–23	v	v	v
C. *X subject only to measurement error, or free of error; parental populations various*				
(a) No error in X; Y subject to measurement error or natural variability or both	26	b	b	b
(b) X with error, its values "controlled"; Y as in (a)	27,28	b	–	b
(c) X with error, its values "modified controlled"; Y as in (a)	27,28	b	–	b (approx.)
(d) X and Y with measurement error only, their values not controlled; supplementary information on λ available	29	b	d	h
(e) Same as (d), but λ unknown	30–34	b	d	v, k, or n
D. *Both X and Y subject to natural variability and to measurement error*				
(a) Random sample from a bivariate normal population	30–37	b	d	v, k, or n
(b) Non-random but symmetrical sample from an open-ended population	30–37	v, k, or n	v, k, or n	v, k, or n

the order of w. In spite of the fact that the correlation is strong, this figure is less even than the predictive regression 1.87, hence it cannot possibly be the best estimate (section 29). The last two estimates in the list above are both possible, but since the variability in the series must be natural for the most part, the GM functional estimate is best. The corresponding relationship is:

$$F = 12.1 + 2.12w$$

This functional expression should also be used for predictions, since the series is open-ended.

Discussion

In the past the distinction between natural variability and measurement error has not been sufficiently appreciated. It is of fundamental importance in assessing what regression to use. Almost as important is the nature of the frequency distribution under consideration: a statistic developed for normal distributions does not necessarily apply to non-normal ones. Also, as Berkson has shown, the manner in which observations are obtained can affect the choice of the statistic to be used. All these things need to be understood by the working scientist if he is to make the most appropriate quantitative generalizations from his data.

While this paper considers straight lines only, it is obvious that many of the points raised would have a bearing on fitting quadratic or higher-degree expressions, or multiple regressions. The theoretical aspects of this can become quite complex, particularly when there is no objective reason for selecting any one of a set of variates as a dependent variable.

In addition to ordinary line fitting, the considerations discussed above have an important bearing on the use of analysis of covariance, which of course involves predictive regressions. For example, the comparison between age-specific and overall weight: length relationships described in Section 49 would ordinarily be done by analysis of covariance. However a "significant" variance ratio (F) for the comparison between the predictive regression coefficients at individual ages and the overall regression would not necessarily indicate a real difference (that is, a difference in *functional* regression) between the two categories.

For the very large class of "open-ended" non-normal distributions it appears that the functional regression should be used routinely both for description and for prediction, instead of the ordinary predictive regression that has been almost universal up to now. This represents a far-reaching change in research procedure, applicable throughout the whole of biology and beyond. Would it not be better to stick with the predictive regressions so as to maintain comparability?

I believe the answer has to be no. The point is that already the predictive regressions are not comparable among themselves. A regression statistic computed for each of several open ended populations will in general be based on data having different variances from the regression line, or differen tranges, or both. Either of these introduces a systematic bias into a comparison between predictive regressions, so that part or all of any observed difference may be an artifact. Only the functional regression has the same status for all populations and will permit valid comparisons.

Summary

Table 8 provides a quick reference to the kinds of regressions available and the circumstances in which they can be used. The symbols and references are as follows:

	Symbol	Formula
Predictive regression of Y on X	b	(2)
Predictive regression of X on Y	d	(11)
Geometric mean functional regression	v	(16)
General least-squares functional regression	h	(26)
Wald's functional regression, computed symmetrically (Section 36)	k	(30)
Nair-Bartlett functional regression, computed symmetrically	n	(32)

Notice that the predictive regressions sometimes are also functional, and that a functional regression should sometimes be used for prediction.

The choice between the GM regression (v) and one of the AM regressions (k or n) is a matter of personal judgment, guided by the relative importance of natural variability and measurement error in the data. Use the GM figure if the variability is mostly natural or if the relative accuracy in measuring X and Y is about the same (that is, if the measurement variances of X and Y are more or less proportional to their overall variances). Also the GM regression is usually preferable anyway if the number of observations is small. If it is necessary to avoid such decisions the best rule is to use the GM regression in all mixed situations. It has the additional advantage of involving the simplest computations.

As between the Wald version of the AM regression (k) and the Nair–Bartlett version (n), use k when the number of pairs of variates is small, and n when it is large. The division point would be 15 to 25, depending on the variability.

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Influence of Temperature on Energy Utilization of Embryonic and Prolarval Tautog, *Tautoga onitis*

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LAURENCE, G. C. 1973. Influence of temperature on energy utilization of embryonic and prolarval tautog, *Tautoga onitis*. J. Fish. Res. Board Can. 30: 435–442.

When daily energy budgets were calculated by two methods for tautog, *Tautoga onitis*, from fertilization to complete yolk absorption at three temperatures (16, 19, and 22 C), neither method indicated a deficiency of yolk energy for growth and metabolism prior to exogenous feeding capability. Deficits were indicated on the day of feeding capability at all temperatures by the oxygen consumption method, the more precise but less inclusive of the two. The dry weight method, which gave the more conservative estimates, indicated no deficit for the entire period of yolk absorption at 16 C, a deficit one day after feeding capability at 19 C, and a deficit on the day of feeding capability at 22 C.

Regression analysis of oxygen consumption indicated that 22 C may be in the upper temperature limits for normal metabolism by embryonic and prolarval tautog. Yolk utilization efficiencies and postlarval size at complete yolk absorption tended to be inversely related to temperature. The ecological implications and the effects of temperature on the critical timing involved in the interactions of feeding capability, availability of prey and occurrence of energy deficits are discussed.

LAURENCE, G. C. 1973. Influence of temperature on energy utilization of embryonic and prolarval tautog, *Tautoga onitis*. J. Fish. Res. Board Can. 30: 435–442.

Nous avons calculé par deux méthodes les budgets énergétiques quotidiens du tautogue noir, *Tautoga onitis*, depuis la fécondation jusqu'à l'absorption complète du vitellus, à trois températures (16, 19, et 22 C). Ces méthodes (consommation d'oxygène et poids sec) ont été établies en vue de déterminer si l'énergie disponible dans l'embryon et dans les réserves vitellines prolarvaires suffisaient aux exigences de la croissance et du métabolisme durant la période d'absorption du vitellus. Ni l'une ni l'autre des méthodes n'indique un déficit d'énergie vitelline avant que le poisson ne commence à se nourrir activement, ce qui se produit, à chaque température, avant absorption complète du vitellus. Nous avons calculé les déficits au moment ou apparaît l'aptitude à se nourrir à toute température par la méthode de consommation d'oxygène; par ailleurs, la méthode du poids sec ne prédit aucun déficit énergétique durant toute la période d'absorption du vitellus à 16 C, un déficit une journée après apparition de l'aptitude à se nourrir à 19 C, et un déficit coïncidant avec l'aptitude à se nourrir à 22 C. Une comparaison des deux méthodes indique que la méthode du poids sec, moins précise mais plus universelle, peut prédire des estimations plus conservatrices de l'énergie totale utilisée que la méthode de la consommation d'oxygène, plus précise mais moins générale.

L'analyse de régression de la consommation d'oxygène indique que 22 C est possiblement près des limites supérieures de température de métabolisme normal du tautogue noir embryonnaire et prolarvaire. L'efficacité d'utilisation du vitellus et la taille de la larve au moment de l'absorption complète du vitellus ont tendance à être inversement reliées à la température. Nous examinons les implications écologiques et les effets de la température sur le réglage critique impliqué dans l'interaction de l'aptitude à se nourrir, la disponibilité des proies et la présence de déficits énergétiques.

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THE purpose of this research was to examine the effects of temperature on the energy utilization of embryonic and prolarval tautog (*Tautoga onitis*). The experimental temperatures were 16, 19, and 22 C which encompass the range tautog embryos and larvae normally encounter in nature (Herman 1963). The efficiency with which yolk is transformed into body tissue and the effect of temperature on yolk utilization probably is an important determinant of early survival since size and condition

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of larvae will affect their ability to begin feeding (Braum 1967). Presumably, larger larvae produced by more efficient use of endogenous yolk reserves will have an initial advantage over smaller larvae in foraging ability.

In this study, daily energy budgets were calculated at each temperature from the time of fertilization to complete yolk absorption to compare the energy available in the yolk with that utilized during development. The energy available to an embryo or prolarva was calculated from the caloric value of yolk present during a time interval. The energy expended during development for growth and metabolism was determined by the dry-weight and oxygen-consumption methods.

Materials and Methods

Developing embryos were obtained from ova naturally fertilized in the 39,000 liter experimental aquarium of the Narragansett Laboratory. They were collected with a ½-m plankton net (approximately one hour after fertilization) on three separate occasions from unknown females in the same group of spawning fish. Fertilization time was determined by observing fish in the spawning act. After collection, approximately 1000 embryos per aquarium, were placed in natural sea water (29–31‰ salinity) filtered to remove plankton in 64 liter all glass aquaria where they developed at 16, 19, and 22 C, respectively. Rearing temperatures were maintained within ± 0.5 C of the desired temperature by controlling room temperatures which housed the experimental aquaria.

Analysis revealed that the initial sizes of the newly fertilized embryos were statistically equivalent. T-tests to compare means of initial yolk volumes of the three groups of embryos demonstrated no significant differences at $P=0.05$ ($t = 1.207$ for 16 and 19 C; $t = 1.996$ for 19 and 22 C; and $t = 1.964$ for 16 and 22 C; $t(0.05) = 2.101$, 18 df).

Prolarval capability to feed was determined when microscopic examination showed 50% of a daily sample of 10 individuals possessed fully pigmented eyes, functional mouth and complete alimentary tract. Previous rearing studies of tautog at the Narragansett Laboratory showed prolarvae were able to feed when this particular state of morphological development was attained (C. R. Rogers personal communication).

Samples of 10 embryos or prolarvae at each temperature were collected daily for dry weight determinations. The embryos or prolarvae were rinsed in distilled water and pipetted onto a glass petri dish. They were dried to a constant weight at 90 C for 20–24 hr and desiccated in a silica-gel desiccator. Dry weight of each individual embryo or prolarva was then determined to 0.1 μg on a Cahn Gram electrobalance, and an average was calculated. Embryo dry weight included the weight of the chorion. Samples were ashed in an electric furnace at 550 C for 6 hr to determine of ash-free dry weights.

To estimate yolk volumes, diameters of 10 yolk-sacs were measured daily with a filar ocular micrometer under 20× magnification. The yolk of newly fertilized ova initially was spherical and its volume was calculated from the formula for the volume of a sphere. Twenty four hours after fertilization at 22 C and 48 hr after fertilization at 16 and 19 C, the yolk became eliptical and its volume was calculated from the formula for a prolate spheroid:

$$V = (\pi/6)\ LH^2$$

where L is the length and H is the height of the yolk-sac (Blaxter and Hempel 1963).

Calculations of daily energy budgets were based on the procedures of Toetz (1966) and Laurence (1969). The former (Dry-weight Method) compared the available yolk energy at the beginning of each day with the daily energy loss calculated from the dry weight loss of the entire yolk–embryo/prolarva system. The latter method (Oxygen-consumption Method) compared the daily available yolk energy with the sum of the energies of growth increment and metabolism (oxygen consumption).

Ash-free caloric equivalent (cal/ash-free g) for yolk was determined from the average value of three samples of newly fertilized (1 hr) embryos combusted in a Parr semi-micro oxygen bomb colorimeter. As the embryos and prolarvae utilized the yolk, the caloric equivalent was multiplied by the daily remaining ash-free yolk weight for an estimate of daily available yolk energy. The validity of using the same caloric equivalent for yolk during the entire yolk-sac period was demonstrated by Lasker (1962). He showed that the relative chemical composition of the yolk does not change appreciably during embryonic and prolarval development from the initial composition of an unfertilized ovum. Ash-free caloric equivalent (cal/ash-free g) for larval tissue was determined by combusting three samples of postlarvae that had absorbed all their yolk and averaging the individual determinations. Daily energy values for larval tissue and growth increments were computed by multiplying the caloric equivalent for larval tissue by the daily ash-free prolarval weight (minus yolk weight).

The average percent ash of three samples of newly fertilized dry ova was 9.5%. The average caloric value of three samples of newly fertilized dry ova corrected for percent ash yielded a value of 6085.3 ± 264.5 cal/ash-free g. The average ash value for three samples of yolk-free dry larval tissue was 5.1%, and the average caloric value for dry larval tissue corrected for the percent ash was 5119.4 ± 180.1 cal/ash-free g.

Standard manometer equipment (Warburg respirometers) and techniques (Umbreit, Burris and Stauffer 1964) were used to measure oxygen consumption by embryos and prolarvae. Measurements were made daily from 24 hr after fertilization until yolk was completely absorbed. Daily samples for consumption measurements at each temperature consisted of four replicates of 30, 20, or 10 embryos or prolarvae, the number in sample decreasing with increasing age. Temperatures were

maintained within ± 0.5 C of the rearing temperature. Embryos or prolarvae were allowed 1 hr to adjust to the respirometer flasks, and the experimental trials lasted for 2 hr. Average 1-hr consumption values for an individual were calculated from the 2-hr experimental trials. The oxycaloric equivalent of 0.005 (Swift and French 1954) was multiplied by the average consumption values to obtain energy values in calories for metabolism. Periodic slight agitation of the flasks was maintained throughout the trials. It is unlikely that flask agitation subjected the embryos and prolarvae to any more turbulence than normally encountered in the surface layers of pelagic waters.

Yolk utilization efficiencies for a time interval were calculated by dividing the energy value of growth during the interval by the energy value of yolk available in the preceding interval.

Results

Neither of the methods indicated an energy deficit prior to feeding capability at any temperature (Table 1, columns G and J; Table 2, columns F and G). At 22 C, both of the methods indicated a deficit on the day of feeding capability. At 19 C the dry-weight method indicated a deficit 1 day after feeding capability, and the oxygen-consumption method showed a deficit occurring on the day of feeding capability. At 16 C, no deficit for the entire yolk-sac period was calculated by the dry-weight method, whereas the oxygen-consumption method implied a deficit the day of feeding capability.

Larval growth expressed as daily ash-free dry weight, ash free dry weight increments, and energy values was variable and sometimes negative (Table 2, columns C–E) at all temperatures. However, the largest postlarvae at complete yolk absorption were those that developed at 16 C while the smallest developed at 22 C.

Daily oxygen consumption computed by multiplying the averaged one hour experimental values by 24 increased with temperature and age of an embryo or prolarva, as was expected (Table 2, column A), although it was more variable at 22 C than at 16 and 19 C. Least squares regression analysis of the relationship between the logarithm of average hourly oxygen consumption and age at the three temperatures showed that the slopes of the regression equations for 16 and 19 C were similar indicating similar increases in rates of consumption with age, while the slope for 22 C was lower indicating a lower rate of consumption increase with age. (Fig. 1)

Daily yolk utilization efficiencies showed a general upward trend to complete yolk absorption for 16 C and variable trends with a final decrease at complete yolk absorption for 19 and 22 C (Table 3).

Discussion

The term "deficit" refers only to the calculated energy budgets. To interpret the term biologically one must examine it in terms of the influence of temperature on the critical timing and interrelationships of feeding initiation, availability of prey, and utilization of yolk. An actual energy deficit would occur when the larvae were not morphologically or behaviorally capable of capturing planktonic prey. A potential energy deficit might occur when the larvae had developed the ability to feed but encountered food limited in supply or contagious in distribution. Neither of the methods indicated an actual deficit prior to feeding capability at any temperature, in which case it would have been impossible for larvae to offset the deficit by capturing external food. Most of the calculated deficits were potential and occurred the day of feeding initiation, when the prolarvae had at least the capability of meeting the deficit through consumption of food. Larvae with a longer period of time to encounter prey limited in supply or distribution might have an advantage over those with a shorter time interval until an energy deficit. Results of the energy budget analyses of the two methods indicate tautog prolarvae at 16 C either don't encounter an energy deficit or have a longer period from hatching to a potential energy deficit than those at 19 or 22 C.

Differences were noted in the timing of energy deficits indicated by the two methods. Theoretically neither method is more advantageous to use than the other. The dry-weight method is an indirect and the less precise method of measuring metabolic processes. It is the more inclusive, however, since it measures total energy loss of the system including that due to excretion (the method does not allow the extraction of estimates for energy of excretion, however). The oxygen-consumption method is less inclusive but more precise because it directly measures the energy of metabolism (oxygen consumption) but it does not include energy loss of excretion. In view of the results of this research, total energy utilization calculated by the dry-weight method appears to be more conservative than that calculated by the oxygen-consumption method. Also, energy utilization due to metabolism is apparently underestimated by the dry-weight method in comparison with the direct determination of the oxygen-consumption method. The differences are undoubtedly due to residual error associated with methodology and inherent in the operation of the experimental apparatus used in the studies.

The relation between the rate of oxygen consumption and time after fertilization (Fig. 1) suggested that 22 C may be near the upper temperature

TABLE 1. Average daily energy budgets for tautog embryos and prolarvae at three temperatures using the ash-free dry weight method. Each value based on 10 embryos or prolarvae.

		Dry weight (*mg*)			Tissue dry weight (*mg*)		Energy (*cal*)			
Time interval (*days*)	A Percent yolk remaining[a]	B Embryo or prolarva	C Yolk [Col. A × initial value of Col B]	D Yolk ash-free [Col. C × 90.5%[b]]	E Larval [Col. B − Col. C]	F Larval ash-free [Col. E × 94.9%[b]]	G Yolk [Col. D × 6085.3 cal/ash-free g[b]]	H Embryo or prolarva [Col. F × 5119.4 cal/ash-free g[b]]	I Total [Col. G + Col. H]	J Loss [Successive substraction, Col. I]
					16 C					
0	100	29.2	29.2	26.4	0	0	0.160	0	0.160	0
1	82.9	24.7	24.2	21.9	0.5	0.5	0.133	0.003	0.136	0.024
2	63.3	21.9	18.5	16.7	3.4	3.2	0.102	0.016	0.118	0.018
3	48.4	17.3	14.1	12.8	3.2	3.0	0.078	0.015	0.093	0.025
4[c]	40.4	14.9	11.8	10.7	3.1	2.9	0.065	0.015	0.080	0.013
5	17.3	13.5	5.1	4.6	8.4	8.0	0.028	0.041	0.069	0.011
6[d]	5.1	12.4	1.5	1.4	10.9	10.3	0.009	0.053	0.062	0.007
7	0	11.9	0	0	11.9	11.3	0	0.058	0.058	0.004
					19 C					
0	100	34.1	34.1	30.9	0	0	0.188	0	0.188	0
1	83.2	31.6	28.4	25.7	3.2	3.0	0.156	0.015	0.171	0.017
2	68.1	28.5	23.2	21.0	5.3	5.0	0.128	0.026	0.154	0.017
3[c]	24.6	14.8	8.4	7.6	6.4	6.1	0.046	0.031	0.077	0.077
4[d]	2.7	11.9	0.9	0.8	11.0	10.4	0.005	0.053	0.058	0.019
5	0	9.8	0	0	9.8	9.3	0	0.048	0.048	0.010[e]
					22 C					
0	100	27.4	27.4	24.8	0	0	0.151	0	0.151	0
1	52.1	18.9	14.3	12.9	4.6	4.4	0.079	0.023	0.102	0.049
2[c]	23.3	10.6	6.4	5.8	4.2	4.0	0.035	0.020	0.055	0.047
3[c]	1.5	9.2	1.2	1.1	8.0	7.6	0.007	0.039	0.046	0.009
4[d]	0	8.1	0	0	8.1	7.7	0	0.039	0.039	0.007[e]

[a]Initial volumes: 0.508 mm³ for 16 C, 0.523 mm³ for 19 C, and 0.493 mm³ for 22 C.
[b]See text.
[c]Interval in which hatching occurred. Times to 50% hatch: 85 hr for 16 C, 58 hr for 19 C, and 48 hr for 22 C.
[d]Prolarvae capable of feeding.
[e]Energy deficit.

TABLE 2. Average daily energy budgets for tautog embryos and prolarvae at three temperatures using the oxygen consumption method. Each value for metabolism based on four replicates of 10–30 organisms; for others, on 10 organisms.

	Metabolism		Ash-free dry weight (*mg*)		Energy (*cal*)		
Time interval (*days*)	A Oxygen consumption (μl/larva per day)	B Oxygen consumption energy (*cal*) [Col. A × 0.005[a]]	C Larval	D Larval growth increment [Difference between successive values of Column C]	E Larval growth increment [Col. D × 5119.4 cal/ash-free g[a]]	F Yolk	G Sum for growth and metabolism [Col. B + Col. E]
				16 C			
0	–	–	0	0	0	0.160	0
1	0.240	0.001	0.5	0.5	0.003	0.133	0.004
2	0.720	0.004	3.2	2.7	0.014	0.102	0.018
3	1.104	0.006	3.0	0	0	0.078	0.006
4[b]	3.696	0.018	2.9	0	0	0.065	0.018
5	7.272	0.036	8.0	5.1	0.026	0.028	0.062
6[c]	7.368	0.037	10.3	2.2	0.011	0.009	0.048[d]
7	9.120	0.046	11.3	1.0	0.005	0	0.051[d]
				19 C			
0	–	–	0	–	0	0.188	0
1	0.672	0.003	3.0	3.0	0.015	0.156	0.018
2	1.488	0.007	5.0	2.0	0.010	0.128	0.017
3[b]	2.592	0.013	6.1	1.1	0.006	0.046	0.019
4[c]	6.696	0.033	10.4	4.3	0.022	0.005	0.055[d]
5	14.784	0.739	9.3	0	0	0	0.739[d]
				22 C			
0	–	–	0	–	0	0.151	0
1	1.848	0.009	4.4	4.4	0.023	0.079	0.032
2[b]	2.880	0.014	4.0	0	0	0.035	0.014
3[b]	2.328	0.012	7.6	3.6	0.018	0.007	0.030
4[c]	7.968	0.040	7.7	0.1	0.001	0	0.041[d]

[a]See text.
[b]Interval in which hatching occurred. Times to 50% hatch: 85 hr for 16 C, 58 hr for 19 C, and 48 hr for 22 C.
[c]Prolarvae capable of feeding.
[d]Energy deficit.

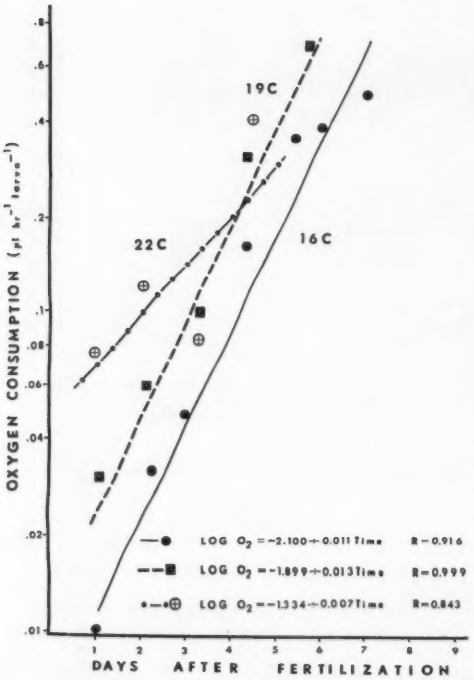
OXYGEN CONSUMPTION (µl hr⁻¹ larva⁻¹)
19C
22C
16C
LOG O2 = −2.100 + 0.011 Time R = 0.916
LOG O2 = −1.899 + 0.013 Time R = 0.999
LOG O2 = −1.334 + 0.007 Time R = 0.843
DAYS AFTER FERTILIZATION

FIG. 1. Relation between average hourly oxygen consumption and age for embryonic and prolarval tautog at 16, 19, and 22 C.

limit for normal metabolism by prolarval tautog. This was indicated by the reduced slope of the regression line at 22 C as compared with those at 16 and 19 C. Fry (1957) demonstrated that active rate of metabolism can decline at the upper limits of temperature tolerance even below the lethal level.

The variation in daily growth and negative growth prior to yolk absorption exhibited by larval tautog at the three temperatures (Table 2, columns C–E) in these experiments is not unusual in view of the results of other researchers. Blaxter (1970) noted for a number of species that development at different temperatures can produce larvae with morphological differences as well as different percentages of yolk and larval tissue at hatching. Full yolk utilization is often incomplete when maximum prolarval tissue weight is reached for a variety of species with different initial egg sizes (Blaxter and Hempel 1963; Laurence 1969; Marr 1966). Farris (1959) separated growth in length of four pelagic, marine species into several categories including a slow, variable phase near yolk absorption and a subsequent negative phase.

The efficiency with which yolk is transformed to larval tissue and the ultimate size attained by a postlarva at complete yolk absorption can have important effects on eventual survival (Blaxter and Hempel 1963). Larger larvae produced from a more efficient use of endogenous yolk reserves may be more successful than smaller larvae in capturing prey. The largest postlarvae at yolk absorption in this study occurred at 16 C with the smallest resulting at 22 C (Tables 1 and 2). Efficiency of yolk utilization for the entire period of yolk absorption, calculated by dividing the final energy value of larval tissue by the initial value of yolk tissue (Table 1, columns G and H), showed the highest efficiency of 36.3% at 16 C and lower efficiencies of 25.5% and 25.8% at 19 and 22 C, respectively. Daily yolk utilization efficiencies were consistently high in the terminal stages of yolk absorption for 16 C and decreased at yolk absorption for 19 and 22 C (Table 3). The overall

TABLE 3. Daily efficiencies of yolk utilization by embryonic and prolarval tautog at three temperatures. Each value based on 10 organisms.

Time interval (*days*)	Yolk energy (*cal*)	Larval growth increment energy (*cal*)	Yolk utilization efficiency (%)
		16 C	
0	0.160	0	0
1	0.133	0.003	1.9
2	0.102	0.014	10.5
3	0.078	0	0
4	0.065	0	0
5	0.028	0.026	40.0
6	0.009	0.011	39.2
7	0	0.005	55.6
		19 C	
0	0.188	0	0
1	0.156	0.015	8.0
2	0.128	0.010	6.4
3	0.046	0.006	4.6
4	0.005	0.022	47.8
5	0	0	0
		22 C	
0	0.151	0	0
1	0.079	0.023	15.2
2	0.035	0	0
3	0.007	0.018	51.4
4	0	0.001	14.2

trend was for larger tautog postlarvae at yolk absorption and higher yolk utilization efficiency in the lower segment of the temperature range.

The efficiencies of yolk utilization for tautog determined in this research are among the lowest recorded. Yolk utilization efficiencies reported in the literature ranged from 35% for the European plaice (*Pleuronectes platessa*) (Ryland and Nichols 1967) to 77% for the Pacific sardine (Lasker 1962) with many values intermediate (Gray 1926; Hayes 1949; Blaxter and Hempel 1966; Laurence 1969). It is felt, however, that the accuracy in determining the efficiencies in this investigation were high because ash-free dry weights were used rather than the simple dry weights often employed in previous studies. Temperature can cause variable trends in sizes at hatching and in yolk utilization efficiencies of different species. Blaxter (1970) compiled data for a number of freshwater and marine species which indicated that maximum lengths and weights at hatching were attained at the lower end of the specific temperature range for each species. However, Lasker (1964) found the maximum length at hatching of Pacific sardine prolarave at intermediate temperatures. Alderdice and Forrester (1971) noted that the largest prolarvae at hatching of the petrale sole (*Eopsetta jordani*) developed at higher temperatures, although the largest number of viable postlarvae remaining at complete yolk absorption occurred at intermediate temperatures.

Except for this study, the only attempt at calculating energy budgets that implied an actual or potential energy deficit prior to or coincident with the capability of external feeding was the work of Lasker (1962) with the Pacific sardine. Gray (1926), Smith (1947), Tœtz (1966) and Laurence (1969) working with brown trout (*Salmo trutta*), rainbow trout (*Salmo gairdneri*), bluegill (*Lepomis macrochirus*), and largemouth bass (*Micropterus salmoides*), respectively, all noted deficits in the stage of yolk absorption after feeding initiation. It is note worthy that tautog and Pacific sardine larvae, which exhibit possible energy deficits, are from relatively fecund fish for their size which produce pelagic embryos and prolarvae; while the species showing no deficit are fish with reasonably low fecundity and with demersal embryos and prolarvae. The implication is that mechanisms of embryonic and larval survival in the species studied thus far may be more loosely controlled in highly fecund fish which produce pelagic embryos.

In conclusion, these experiments demonstrated that tautog prolarvae may encounter a potential energy deficit at the higher temperatures (19 and 22 C) in their spawning range. Larvae produced in the lower range of spawning temperatures (16 C) do not appear to be as susceptible to a potential energy deficit as those produced at the higher temperatures, are larger at completion of yolk absorption, and have a longer period between hatching and the initiation of feeding or occurrence of a potential energy deficit to encounter planktonic prey that may be limited in distribution or abundance.

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Effects of a Parasitic Gut Nematode on Consumption and Growth in Juvenile Rainbow Trout (*Salmo gairdneri*)

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HISCOX, J. I., AND R. W. BROCKSEN. 1973. Effects of a parasitic gut nematode on consumption and growth in juvenile rainbow trout (*Salmo gairdneri*). J. Fish. Res. Board Can. 30: 443–450.

A technique was developed for differentially infecting juvenile rainbow trout, *Salmo gairdneri*, by introduction of the parasitic gut nematode, *Bulbodacnitis ampullastoma*. Desired infection levels were induced in trout with 80% success. *B. ampullastoma* affected growth and food consumption of trout in the laboratory. The effects of the parasite were evident at low and high ration levels. At low and starvation ration levels the parasite effected a withdrawal of the energy stores of the fish. At higher ration levels, the direct effects of the parasite were compounded by apparent parasite interference with food assimilation by the fish.

B. ampullastoma was directly infective to a warmwater fish, *Lepomis cyanellus*.

HISCOX, J. I., AND R. W. BROCKSEN. 1973. Effects of a parasitic gut nematode on consumption and growth in juvenile rainbow trout (*Salmo gairdneri*). J. Fish. Res. Board Can. 30: 443–450.

Nous avons mis au point une méthode d'infection différentielle de jeunes truites arc-en-ciel, *Salmo gairdneri*, par le nématode parasite du canal alimentaire, *Bulbodacnitis ampullastoma*. Nous avons réussi le palier d'infestation désiré dans 80% des cas. *B. ampullastoma* affecte la croissance et la consommation des aliments de la truite en laboratoire. Les effets du parasite sont évidents à bas et hauts niveaux d'alimentation. A de bas niveaux et à des niveaux de famine, le parasite puise à même les réserves d'énergie du poisson. A des paliers alimentaires plus élevés, les effets directs du parasite se compliquent du fait que le nématode semble nuire à l'assimilation de la nourriture par le poisson.

B. ampullastoma peut infester directement un poisson d'eau chaude, *Lepomis cyanellus*.

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THE effects which parasites have on the metabolism or nutritional state of fish have not been extensively studied. Workers in parasitology have, in the past, concentrated on descriptive taxonomy, while fisheries biologists have concentrated on the description of infection symptoms in parasitized fish. Large parasites and parasites which induce massive effects on their hosts (whirling disease, bass tapeworm, yellow grub disease) have received the most attention with respect to establishment of parasite life cycles, feeding habits, and effects on the fish host. Small endoparasites have received less study in these respects, but recent work may indicate an increased interest. Platzer and Adams (1967) and Moravec (1970) worked with nematode endoparasites of fish and attempted to document their life histories. Dogiel et al. (1961) quantified changes in liver fat and changes in the amounts of Vitamin A stored in the livers of marine cod infected with the larvae of *Contracoecum aduncum*. However, we have been unable to find any bioenergetic studies dealing with effects of nematode endoparasites on growth or food consumption in freshwater fish.

Trout are known to reduce food consumption during reproduction, and to metabolize adipose stores in the liver, pyloric cecae, and on connective tissue (Calhoun 1966; Hoar and Randall 1969). The pyloric cecae are thought to be primary sites of fat absorption, in addition to having enzymatic functions (Lagler et al. 1962; Hoar and Randall 1969). The pyloric cecae are also known to be the primary site of attachment by the parasitic gut nematode *Bulbodacnitis ampullastoma* (Maggenti 1971). The occurrence of *B. ampullastoma* inside the cecal sacs in reproductive fish may reflect

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an attempt by the parasite to utilize the fat stores concentrated in those regions.

The life cycle of *B. ampullastoma* is not known (Eaken et al. 1954; Hoffman 1967); based on characteristics of the order to which it belongs (as given by Chitwood 1950), it probably includes five life stages with one intermediate host, the third usually being the fish-infecting stage. It is probable that *B. ampullastoma* requires a copepod intermediate host similar to that described by Platzer and Adams (1967) for *Philonema oncorhynchi*. Platzer and Adams hypothesize that reproduction in *P. oncorhynchi* was correlated with that of its host, the sockeye salmon, by a hormonal mechanism. *B. ampullastoma* was described by Maggenti (1971), as was the species most closely resembling *B. ampullastoma*, *B. truttae*.

We used juvenile rainbow trout, which we differentially infected with *B. ampullastoma* taken from naturally infected mature rainbow trout. Since little is known about the parasite, much of our research has involved development of techniques for working with and holding the parasite outside of the fish host. Consumption and growth experiments were conducted on test fish as the first step in evaluating the effects of the parasite on its host. A quantification of consumption and growth as related to parasite load was the focal point of this effort.

Methods and Materials

Experimental Animals

The parasites, *B. ampullastoma*, were taken from 2- and 3-year-old, naturally infected brood rainbow trout, *S. gairdneri*, of the Kamloops and Mt. Whitney strains. Male and female brood trout were obtained from the California Department of Fish and Game Hot Creek Hatchery. The fish were 46–66 cm long, mostly in reproductive or postreproductive state, and all in good condition. Up to 84 *B. ampullastoma* were found in the region of, and within, the pyloric ceca, highest numbers being found in nongravid fish. Reproducing parasites were found only in reproductively ripe trout, even though nongravid fish, on the average, contained more parasites than gravid fish.

Feeding habits of *B. ampullastoma* in the host fish were not determined. In most fish, the parasites were attached by mouth to the mucosal layer of the gut, but were occasionally found motile in the gut fluid; they were found only in the anterior third of the cecal region, just behind the stomach. They were often in clusters around the pyloric sphincter or around openings of the cecal sacs into the gut. In reproductively ripe brood fish, the gut had atrophied somewhat, and *B. ampullastoma* were found inside the cecal sacs; however, no parasites were found attached in this region in nonripe fish.

Juvenile *S. gairdnerii* were obtained from the California Department of Fish and Game American River Hatchery, and ranged from 8.9 to 14.8 cm in length, were less than a year old, and were in good condition. The trout possessed high levels of fat deposited around the liver and ceca. No intrinsic parasites were found in the fish.

Stocks of brood trout and juvenile trout were held separately in large tanks and fed twice daily to repletion on Rangens Brood Trout Pellets. During the experiments the juvenile trout were kept in 15-gal glass aquaria divided into chambers of 0.3 m^3, one to each chamber. Water temperature was controlled with two refrigeration pump units and was maintained at 13.2 C, fluctuating no more than 0.8 C during any test. Dissolved oxygen values ranged from 7.0 to 9.3 mg/liter. Water was filtered with air-driven, glasswool, and activated charcoal filters. All test fish were dipped in malachite green disinfectant before being used. No fish were found with fungus or bacterial infections.

Infection Techniques

An acceptable infection technique was defined as that which would allow induction of a predictable number of parasites in individuals of any group of test fish. The minimum level indicative of an acceptable technique was set at 75% infection success (three out of every four introduced parasites to be attached in a test fish and present on later examination). In development of an infection technique, the following variables were tested singly and in combination for effect on infection success:

1, Size and sex of test fish and parasite; 2, Mechanical and chemical removal of attached parasites from fish mucosa; 3, Methods for introducing the parasite; 4, Methods for holding the parasite for extended periods in vitro; 5, Critical attachment time and response of the parasite to anesthetic used.

No experiments were designed to test the first variable, but lengths of all fish and parasites were measured before and after most experiments. All test fish were sexed on termination of an experiment; the nematodes were also sexed whenever possible, as dictated by size and maturity.

Methods attempted for removing *B. ampullastoma* from the cecal mucosa of brood trout included removal of the nematode with forceps and use of chemical "releasing agents." The dissected cecal region was washed with an agent before the nematodes were extracted, and chemicals were judged on ability to disperse the mucosal lining without killing the parasite. Hydrochloric acid and sulfuric acid, (0.01–0.1 M) and sodium bicarbonate (2.5–100 mg/liter distilled water) were tested.

The most successful method for introducing the parasite into the test fish stomach was by use of a 20-ml medical syringe fitted with polyethylene tubing 12 cm long and 0.9 mm in diameter. The parasites were placed in one-half of a Libby #4 gelatin capsule containing a drop of 0.7% saline solution, the open end was sealed with gelatin, the capsule was placed in the syringe tube, which was passed through the fish esophagus, and the capsule was ejected into the stomach. A modifica-

tion involved introduction of the parasites without use of gelatin capsules.

Several saline solutions (0.5%–1.0% NaCl in distilled water) and Alsevers solution (prepared as follows, anhydrous dextrose — 41.0 g, sodium citrate — 16.0 g, sodium chloride — 8.4 g, and distilled water — 2.0 liters) were tested for ability to maintain 5, 10, 15, and 20 *B. ampullastoma* in covered petri dishes for 14 days at 13 C. *p*H was adjusted to 6.1 with a 10% citric acid solution. Tricaine methanesulfonate (M.S.222) was used to anesthetize the fish (Bell 1964). The critical attachment time of the parasite in anesthetized and nonanesthetized fish was determined by killing test fish at intervals after infection and checking for attached parasites.

Growth and Consumption

In all growth and food consumption experiments, test fish infection was by the syringe/capsule technique. *B. ampullastoma* were washed from the cecal region of the brood trout with a dilute $NaHCO_3$ solution (50 g/liter H_2O) and motile nematodes were collected with an eyedropper. The parasites were kept at 13 C in Alsevers solution. Fish were anesthetized with M.S. 222. Test fish were starved 36 hr before being weighed. All weights were taken without parasites. Tests were not begun until 12 hr after introduction of nematodes. Fish were fed weighed amounts of Rangens Brood Trout Pellets (3/32 inch short pellet; 95% dry by weight). Dry weight conversion factors were used to determine the original dry weight of all test fish; a separate factor was determined for each experiment. The test conditions are summarized in Table 1.

Host Specificity

A host-specificity experiment was conducted with *B. ampullastoma* from trout and available nonsalmonid fish genera; success was measured by nematode attachment. The infection technique involved syringe introduction of nematodes without the gelatin capsule, and four to six nematodes were introduced into each fish. All portions of the fish's alimentary canal were checked for the parasite after 48 hr. Water temperature was optimized for each fish species (Calhoun 1966) and never varied more than 0.7 C for a given species. The following fish species used in this experiment were collected by seine in Yolo County, California: *Cyprinus carpio*, European carp; *Pomoxis nigromaculatus*, black crappie; *Lepomis macrochirus*, bluegill sunfish; *Lepomis cyanellus*; green sunfish; *Morone saxatilis*, striped bass; and *Micropterus salmoides*, largemouth bass.

Results and Interpretation

Infection

The infection technique which proved most successful included use of sodium bicarbonate solution (50 g/liter) for releasing parasites from the mucosa of the pyloric cecae, Alsevers solution for in vitro maintenance of parasites at 13 C, and the syringe/capsule technique of introduction. This technique generally produced an infection rate of 80%. The lowest infection for the technique was 58.3% and occurred during the starvation experiment. This low value may reflect release of some *B. ampullastoma* and passage out of the starving fish before they could be counted.

Use of sodium bicarbonate as a releasing agent apparently served to reduce mechanical injury to larger nematodes (over 8 mm) collected from brood fish. Efficacy of test fish infection by large nematodes was increased 43% with this technique.

Alsevers solution was best for maintaining live parasites for long periods out of the fish, and was most effective at *p*H 6.1. The 0.7% NaCl solution produced the best results in the saline series. Metabolic waste was not found to influence the numbers of nematodes that could be maintained successfully per petri dish, within the limits of the tests.

The syringe/capsule technique of introduction proved to be only slightly more successful than the same technique without the capsule, but reduced the time required for anesthetization and eliminated regurgitation of the parasites by the trout. Because feeding test fish immediately after parasites were introduced lowered the rate of infection, this practice was eliminated in growth and consumption experiments. The anesthetic (M.S. 222), was found to

TABLE 1. Test conditions for food consumption and growth experiments conducted on juvenile rainbow trout infected with *Bulbodacnitis ampullastoma*.

Experiment	Length of test (*days*)	Ration	No. Trout		Size of Trout (*cm*)	Initial Trout Wet Weights (*g*)	Parasite numbers injected
			Control	Test			
1	14	Repletion	2	6	11.7–12.5	16.0–22.6	1–10
2	10	1%,3%,5%,7% wet wt	4	12	7.9–13.7	13.1–34.0	4–5
3	12	Starvation	4	8	9.4–13.9	12.9–22.1	1–10

TABLE 2. Consumption and growth data for juvenile rainbow trout containing various parasite loads; fish fed to repletion. Growth Experiment 1, 14 days duration.

Fish	No. of parasites		% Dry Wt[a]	Wet wt/l (*g/cm*)	Food eaten (*g dry wt*)	Growth rate (*mg/g/day*)	Consumption rate (*g/g/day*) × 10	Growth efficiency (%)
	Introduced	Recovered						
1	0	0	24.4	1.8	13.6	9.28	2.75	33.8
2	5	4	23.9	1.9	12.9	8.25	2.62	31.5
3	10	6	22.7	2.7	14.0	8.54	2.46	34.7
4	10	10	22.7	2.0	15.1	9.05	2.86	31.6
5	5	1	24.7	1.9	13.7	9.46	2.66	35.5
6	10	8	21.5	2.1	14.8	8.71	3.05	28.6
7	5	5	20.6	1.7	14.0	9.91	3.19	31.0
8	0	0	23.8	1.7	12.9	9.38	2.80	33.5

[a]Dry weight conversion factor equals 24.70%.

have no adverse effects on infection success and was used whenever possible, since it reduced handling time of the juvenile trout. Critical attachment time of *B. ampullastoma* under existing test conditions was between 6 and 8 hr. Infection success was not affected by size or sex of either the parasite or the test fish with the syringe/capsule or syringe-only techniques.

GROWTH AND CONSUMPTION

Results of experiment 1 (Table 1) suggest that an increased consumption rate and a decreased growth rate occur as the parasite load increases (Table 2, Fig. 1); with increased parasite load, final wet weight per centimeter of fish also increased. But as numbers of *B. ampullastoma* were increased, percent dry weight in the test fish decreased.

One could expect parasitized fish to eat more and grow less than nonparasitized fish. Increased

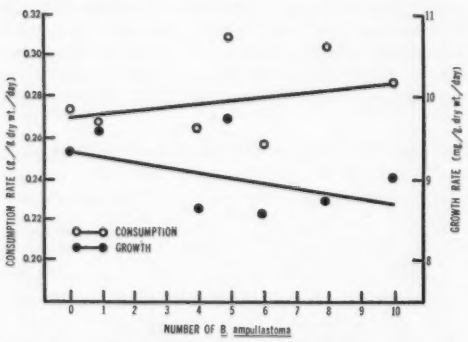

FIG. 1. Relation between trout consumption and parasite load, and trout growth and parasite load. Trout fed to repletion. Growth Experiment 1.

wet weight and decreased dry weight has been documented in fish that mobilize protein or fat stores for energy and take on metabolic water in their place (Hoar and Randall 1969). The unusual factor in our results is that this test was run with a repletion ration. It is highly improbable that *B. ampullastoma* in the numbers present were able to consume enough of the food ingested by the fish to account for the observed differences in consumption, growth, dry weight, and wet weight of the fish. It may be that the parasite affects digestive, absorptive, or energy-conversion processes of the fish, triggering a mobilization of protein and fat stores which in turn results in changes in the percentages of dry and wet weight. The interference may be purely mechanical, with the mass of the parasite reducing the absorptive surfaces of the gut, or may be chemically or hormonally mediated.

In the second experiment (Table 1), consumption in control and parasitized fish was found to be comparable at each ration level (Table 3). Growth efficiencies of control fish were high at each ration level, but were higher at the 1 and 7% levels. Wet weight per centimeter of fish decreased in both the control and parasitized trout with increasing ration, being lower in controls; in contrast, percent dry weight of both control and parasitized fish increased with increasing ration, but controls maintained a higher percentage dry weight at each level. Both the parasitized and control fish exhibited increased growth as more food was made available, but parasitized fish grew at a slower rate than controls at comparable levels (Fig. 2).

These data suggest that *B. ampullastoma* reduces assimilation in juvenile trout. Effects of the parasite were probably accentuated at high ration levels because the dry pelleted food was passed more

TABLE 3. Consumption and growth data for juvenile rainbow trout parasitized at a given level and fed at 1%, 3%, 5%, and 7% of original wet weight. Growth Experiment 2, 10 days duration.

Fish	No. of parasites		Ration (% *wet body wt*)	% Dry wt[a]	Wet wt/l (*g/cm*)	Growth rate (*mg/g/day*)	Consumption rate (*g/g/day*) × 10	Growth efficiency (%)
	Introduced	Recovered						
1	5	4	1	18.5	2.3	1.29	0.45	28.6
2	5	5	1	17.8	2.4	1.27	0.45	28.2
3	0	0	1	21.0	1.8	2.41	0.45	53.2
4	5	5	1	19.2	2.5	–0.17	0.45	–
5	5	5	3	19.7	2.6	3.20	1.35	23.7
6	0	0	3	20.5	2.1	3.37	1.35	25.0
7	5	4	3	18.9	1.9	4.58	1.35	33.9
8	5	4	3	–	–	–	–	–
9	0	0	5	21.8	1.8	12.90	2.20	58.6
10	5	5	5	21.3	2.8	6.11	2.20	27.8
11	5	4	5	21.6	2.1	10.85	2.20	49.3
12	5	5	5	21.9	1.9	13.78	2.20	62.7
13	4	4	7	22.3	2.2	12.76	2.98	42.8
14	5	5	7	20.8	2.3	8.87	2.80	31.6
15	5	5	7	21.9	1.9	13.44	2.99	44.9
16	0	0	7	22.3	1.5	15.14	2.99	5.06

[a]Dry weight conversion factor equals 21.94%.

quickly through the gut than at lower levels. The situation is suggested by the comparable percentage dry weights of parasitiszed and control trout at the 7% level, in that the parasitized fish grew more slowly at that level. At low levels the parasitized fish were apparently forced to mobilize their fat and protein stores to a greater extent than the control trout in order to maintain a standard metabolism. Thus, at the 1% level, the parasitized fish showed a low percentage dry weight while maintaining a growth rate close to that of the controls, within the 10 days of the test; it is questionable that they could exist under such conditions for extended periods. If the parasites were simply consuming enough energy to cause the observed reduction in growth at the 7% level, then the growth rate at the 1% level should be depressed an equal amount, if not more.

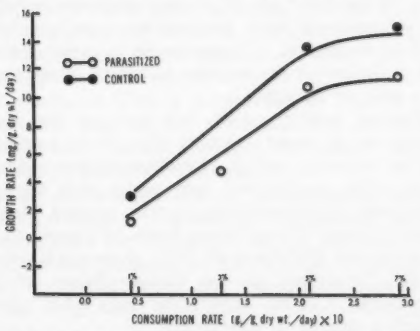

FIG. 2. Relation between percentage dry weight of trout and established consumption rates, using trout with comparable parasite loads and controls. Trout fed at 1%, 3%, 5%, and 7% of wet weight. Growth Experiment 2.

In the final growth experiment (Table 4), parasitized fish were found to die sooner than controls. With only 1–2 nematodes, trout mortality time increased by about 20%, but with 5–7 nematodes it increased by more than 60%, over the controls. The final wet weight was found to increase and percent dry weight to decrease with parasite load, as in previous experiments. The loss of weight in juvenile trout is expressed as a negative growth rate (Fig. 3); the heavily parasitized fish lost weight much faster than the control or lightly parasitized fish. These observations complement the conclusion that at starvation or near-starvation ration levels, presence of *B. ampullastoma* induces an increased mobilization of energy stores in test fish over that in controls. Increased mobilization leads to an earlier death, under these conditions. It is also significant that the parasite effects observed in this test were caused by seven or fewer nematodes.

HOST SPECIFICITY

B. ampullastoma was recovered only in the green sunfish, *Lepomis cyanellus*, of the several nonsalmonid species tested. Parasites were attached in

TABLE 4. Rates of weight loss of juvenile rainbow trout parasitized at various levels and starved until dead. Growth Experiment 3, 12 days duration.

Fish	No. of parasites		Time to death (*hours*)	% Dry wt[a]	Wet wt/l (*g/cm*)	Rate of weight loss (*mg/g/hr*)
	Introduced	Recovered				
1	0	0	236	21.9	1.0	1.99
2	0	0	222	22.9	1.1	1.71
3	5	4	163	18.3	1.5	2.80
4	5	1	196	20.7	1.5	1.53
5	10	6	164	19.1	1.9	3.03
6	10	7	140	18.9	1.8	2.93
7	0	0	280	22.9	1.3	0.61
8	0	0	229	21.2	1.1	1.26
9	5	3	168	20.8	1.4	1.81
10	5	2	198	21.7	1.6	1.48
11	10	6	166	19.3	1.3	2.60
12	10	6	192	18.6	1.7	2.47

[a]The initial percentage dry weight was taken to be 22.1.

four of five green sunfish and were motile in the cecal region of the fifth; of 28 *B. ampullastoma* introduced, 20 were recovered and 16 of these were attached to the mucosal layer of the pyloric cecal region, an analogous location to that which it occupied in nonripe rainbow trout. These observations suggest similarity between the green sunfish and rainbow trout, as concerns the requirements of *B. ampullastoma*.

Discussion

Our results suggest that *B. ampullastoma* has an effect on growth and metabolism in a trout host that is starved or at low ration levels. Under a starvation regime, the parasite load does not have to be heavy to elicit a noticeable degradation in the condition of its host. At higher ration levels, the effect of parasitism may reflect disruptions of the assimilatory processes in the fish.

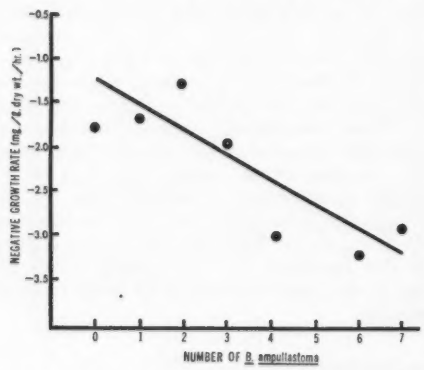

FIG. 3. Relation between rates of weight loss and parasite load in juvenile trout starved until dead. Growth Experiment 3.

B. ampullastoma does establish in wild populations of trout and has been found in several locations in California. Up to 13 of the parasites have been reported in a single "catchable" size fish (A. R. Maggenti personal communication). Several problems emerge if we try to extrapolate the observed results to wild trout populations. Wild trout are not confined in massive numbers during initial development and growth, as are hatchery fish. If the life cycle of *B. ampullastoma* includes an intermediate host, particularly a pelagic host such as a copepod, it is reasonable to expect higher fish infection in a crowded hatchery pond than in a lake or stream.

Further, wild trout do not feed on dry food pellets as do most hatchery fish. It is possible, though unlikely, that *B. ampullastoma* has a significant effect on its host only under such feeding conditions. Hatchery trout usually contain higher levels of body fat and lower levels of protein than do wild trout (Phillips et al. 1957; Hoar and Randall 1969). Depending on the feeding habits of *B. ampullastoma*, these differences may also affect infection success in wild trout.

It would be premature to speculate on the effects of this parasite on its wild host. Under hatchery conditions, however, it appears that effects of the parasite can be significant at any ration level. In

situations where fish are being fed massive amounts of food, growth is going to be closely correlated with assimilation. The fish do not have to expend much energy swimming or procuring food, and most fish are fed near repletion. If a gut parasite is in some way placing limits on assimilation, growth will be affected.

In infected juvenile trout fed at 7% of body weight, growth rate was depressed approximately 4 mg/g dry weight of fish/day in the test fish as compared to the controls at that level. The parasite effect in terms of lost production can be expressed by the formula $P = G \times B$ (Allen 1950) where for a particular time interval: P = production in terms of mass or energy, G = growth rate (usually taken to be constant), and B = mean biomass of the fish population.

The potential biological and economic effects of *B. ampullastoma* can be approximated by extrapolating the observed effects of the parasite to a potentially real hatchery situation. Consider a trout hatchery which contains 20 rearing ponds for "catchable" rainbow trout. The annual production of each pond is between 4000 and 6000 lb of fish, at a cost of $0.95 per pound of yearling fish produced (all figures from California Department of Fish and Game, American River Hatchery; personal communication). Assume that the hatchery has a 50% infection level of *B. ampullastoma*, that parasite load is four or five parasites per infected fish, and that this depresses growth rate by 4 mg/g dry weight of fish/day.

Taking the average dry weight of the hatchery trout to be 23% of wet weight (mean of observed values), then each pond contains approximately 1150 lb dry weight of fish biomass at any given time. Using Allen's formula, the loss in fish production for each pond amounts to 4.60 lb per day. In a year, the 20 ponds lose 16,790 lb of fish production due to the parasite. At $0.95 per lb, this amounts to a cost of $15,950.50, in excess of fish produced. This is a considerable loss for an operation with limited pond and fish infection. Losses would be much greater with even a slight increase in parasite occurrence.

It is also possible that *B. ampullastoma* affects the reproductive potential of parasitized trout. Competition for available energy between the reproductive processes of the fish and its parasite load could well lower the reproductive potential of the trout, with consequent losses to hatchery operation.

At low food levels, or under starvation conditions, *B. ampullastoma* could be expected to elicit responses in wild trout similar to those observed in these tests. The effects of the parasite might be even more pronounced in wild fish due to their lower fat content, indicating a smaller energy store. The stress factor of the parasite might also be compounded by the more frequent environmental fluctuations affecting wild trout, and the higher energy output of such fish to obtain food.

This study establishes *B. ampullastoma* to be a pliable research tool. Using the described techniques, the researcher is provided with a fairly large, easy-to-manipulate parasite that can be introduced and established at discrete infection levels in rainbow trout. As studies of fish parasitology expand to encompass the effects of nematode endoparasites on trout assimilation, respiration, behavior, etc., the demand for a documented and convenient parasite will increase. We believe *B. ampullastoma* to be appropriate for such studies.

Acknowledgments

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NOTES

Food and Inferred Feeding Behavior of Northern Sand Lance (*Ammodytes dubius*)

J. S. Scott

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Scott, J. S. 1973. Food and inferred feeding behavior of northern sand lance (*Ammodytes dubius*). J. Fish. Res. Board Can. 30: 451–454.

Gut contents of northern sand lance (*Ammodytes dubius*) were identified, measured by volume, and compared to volumetric analyses of plankton hauls. *A. dubius* is a plankton feeder, copepods (mainly *Calanus finmarchicus*) constituting about 65% of its food by volume. It combines filter feeding with selective feeding on larger food items such as euphausiids and fish larvae.

Scott, J. S. 1973. Food and inferred feeding behavior of northern sand lance (*Ammodytes dubius*). J. Fish. Res. Board Can. 30: 451–454.

Nous avons identifié les contenus stomacaux d'*Ammodytes dubius*, en avons déterminé le volume et les avons comparés aux analyses volumétriques de prises de plancton. *A. dubius* est planctonophage, les copépodes (principalement *Calanus finmarchicus*) constituant environ 65%, en volume, de son régime. Les proies plus grandes telles euphausides et larves de poissons sont absorbées par filtrage combiné avec alimentation sélective.

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Although the taxonomy and larval characteristics of northwest Atlantic Ammodytidae have received much attention (Einarsson 1951; Richards et al. 1963; Richards 1965; Scott 1968; Winters 1970), their general biology has been largely neglected. Scott (1968, 1972) gave some data on the biology of northern sand lance (*Ammodytes dubius*) from the Nova Scotia Banks. Reay (1970) included west Atlantic species (*A. dubius*, *A. hexapterus*, and *A. marinus*) in a synopsis of data on *Ammodytes* from the North Atlantic illustrating the lack of data on northwest Atlantic as compared to European species.

Recent investigations of various sand lance groups of the northwest Atlantic revealed differences in growth rates between neighboring areas (J. S. Scott in preparation). This suggested isolation of groups and led to a consideration of ecology and behavior of *Ammodytes* that would result in such isolation. Direct observation of behavior of the fish in natural conditions was not possible. However, observations of times and places of occurrence, food, and reported behavior of other *Ammodytes* species suggest a pattern of behavior for *A. dubius* that may explain the variability and unpredictability of sand lance catches on the Scotian Shelf. This unpredictability has discouraged efforts to exploit sand lance as a supplement to herring in the Canadian fish reduction industry. An understanding of the fish's behavior may suggest new techniques to overcome the problem.

Materials and methods — Fish were captured by #36 Yankee trawls, frozen immediately, and thawed later for laboratory examination. For comparison of available plankton with gut contents of the fish, plankton hauls were made with a 6-ft Isaacs–Kidd midwater trawl with #471 Nytex codend and polythene plankton bucket with 40/40 wire-mesh window. At each station sampled, 15-min horizontal tows were made at 2, 20, 40, 100 m, depth permitting. Plankton samples were preserved in 8% formalin in sea water. Large samples were subdivided by plankton divider for estimates of contents.

Stomach and gut contents of 486 individual fish, length 15–31 cm, and 14 plankton tows were examined. Total wet-settled volumes were measured for stomach contents, total plankton hauls, and for each individual class of identifiable food item, after sorting by hand.

Results — Food — Examination of stomach contents of *A. dubius*, taken at various localities

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and seasons, shows that it feeds on a wide variety of planktonic rather than benthic organisms (Table 1). Nearly all fish contained copepods (mainly *Calanus finmarchicus*), the next most frequently occurring items (in order of importance) being various crustacean larvae, invertebrate eggs, polychaete larvae, larvaceans, fish eggs, pteropods, and cirripede larvae. Remaining food items occurred rarely except for phytoplankton, mainly *Coscinodiscus*, which was recorded in 10.85% of stomachs examined. Sand grains were common in all stomachs examined.

Importance of copepods in the diet is more clearly shown by volumetric analysis of stomach contents of 130 specimens (Table 1) taken in May 1968. They comprise the bulk of the food (64.7%) and, together with polychaete larvae, euphausiids, crustacean larvae, and larvaceans, total almost 99% of contents analyzed by volume. Obviously, proportions of seasonally available items such as fish eggs and larvae must vary considerably during the year.

Comparison of volumetric amalyses of stomach contents and plankton hauls, taken at about the same places and times (Table 1), indicates that *A. dubius* is a fairly selective feeder showing a definite preference for the larger zooplankton: copepods, euphausiids, and polychaete larvae. Euphausiids (mainly *Meganyctiphanes norvegica*) were the largest food organisms found, up to a maximum length of 1.5 cm. This indicates the limiting size of food item acceptable to *A. dubius* as larger euphausiids were common in plankton tows.

The nature and size-range of its food and its long gill rakers indicate that *A. dubius* is both a filter feeder for small prey and a selective feeder for larger items such as euphausiids and polychaetes. This combination explains the variety of organisms in the diet.

The comparatively high frequency of phytoplankton was interesting. It was negligible in terms of volume and occurred in only one sample catch but in high concentrations. In European sand lances there is some correlation between frequency of occurrence of *Coscinodiscus*, size of sand lance, and frequency of occurrence of copepods in stomachs, most *Coscinodiscus* and copepods occurring in the smallest lance (Macer 1966). No such correlation was found in Nova Scotia Banks' material. It was considered that *Coscinodiscus* might be undigested remnants of gut contents of sand lance prey, but its common occurrence in European

TABLE 1. Frequency and volumetric analyses of food items of northern sand lance (*Ammodytes dubius*) in stomach contents and plankton hauls. Frequencies are composite from all samples, volumes from synchronous samples of fish and plankton taken on Nova Scotia Banks, May 1967. (Numbers of fish/plankton hauls, in parenthesis).

	% frequency of occurrence (486)	% volume of stomach contents (130)	% volume of plankton tows (14)
Copepods	95.74	64.97	58.88
Crustacean larvae	43.25	2.88	14.48
Invertebrate eggs	34.34	0.40	–
Polychaete larvae	27.75	14.88	3.02
Fish eggs	22.36	0.08	5.96
Larvaceans	21.87	2.56	6.44
Pteropods	20.25	0.05	1.38
Cirripede larvae	15.71	0.29	0.10
Phytoplankton	10.85	0.05	–
Euphausiids	6.16	13.68	3.74
Fish larvae	5.51	0.05	–
Amphipods	3.89	0.08	6.00
Fish remains (scales, etc.)	4.05	0.05	–
Chaetognaths	0.48	"	–
Hydroid	0.32	"	–
Hydromedusae	0.32	"	–
Brachyuran crab	0.32	"	–
Holothuroidean larva	0.16	"	–
Ctenophores	0.16	"	–
Isopods	0.16	"	–
Siphonophores	Trace	"	–
Ostracods	Trace	"	–

lances suggests that it is a normal food item and confirms the filter feeding habit of *Ammodytes*.

The diet of *A. dubius* is very similar to that of the North Sea *A. marinus* (Macer 1966). The related *A. lancea* appears to change from a mixed diet with a preponderance of copepods in early life to a diet almost exclusively fish and annelids in largest fish, although the lengths of largest *A. lancea* (16–20 cm) are much smaller than mean length of *A. dubius* examined.

Ecology and behavior — The most interesting characteristic of the sand lances is their ability to burrow into sand or gravel and remain there for long periods. This has been observed directly in shallow water groups in the Baltic (Kuhlman and Karst 1967). Both burrowing and emergence are extremely rapid, the fish entering and leaving the surface almost vertically at swimming speed. Coastal lance may bury themselves above low-water mark and remain buried as the tide recedes and until it covers the area again; digging them out of sand above low water is the commonest source for lance for bait in Britain (Langham 1971a). European sand lances are reported to burrow when approached by predators and during hours of darkness (Langham 1971b). This habit demands a loose, porous substrate in which respiratory water maintains sufficient oxygen to support life, or some unknown physiological adaptation by the fish.

Behavior of *A. dubius* has not been directly observed but the following observations (from Fisheries Research Board of Canada cruises where no other reference is given) permit a tentative pattern of behavior to be postulated for this species on the Scotian Shelf: (1) *A. dubius* was captured in relatively shallow water, generally in less than 50 fathoms, where the bottom is fine sand; (2) Numbers of sand lance were captured in grabs during bottom sampling operations in sandy areas on the Nova Scotian Banks (Dr L. H. King, Bedford Institute, personal communication); (3) *A. dubius* feeds largely on copepods (Table 1); (4) Fish identified as sand lance were observed swimming just below the surface at night. Several sand lance were captured on different occasions in surface waters at night by Isaacs–Kidd midwater trawls. It is unusual to catch active, adult fish in these nets except where they are abundant; (5) Echo-sounder traces indicate that they go down to the sea bed during daylight hours and rise to near surface at night (Drever and Ellis 1968); and (6) Heavy catches have been made at various times of day and all seasons. Conversely, unpredictable periods occurred when no sand lance were caught even with prolonged effort on known sand-lance ground.

In other areas, Madsen (1963) found a definite relation between tides and *A. marinus* catches in the North Sea. His interpretation was that best catches were made during the strongest part of the tides when most food was being swept into the feeding area. The fish are reported to bury themselves at night and are trawled only during daylight (Macer and Burd 1970).

Field observations on *A. tobianus* by scuba divers in the western Baltic (Kuhlman and Karst 1967) showed that they remained buried at night and then emerged in small groups in early morning. These groups coalesced and then moved to deeper water to feed. In early afternoon they moved back to the rest area in small groups for the night.

By analogy, and considering the foregoing observations, *A. dubius* may be expected to form dense schools on the bottom just before and after feeding periods and, at other times, to be buried in sand or feeding near bottom in daylight and near surface at night.

What determines the feeding periods of *A. dubius* is not yet known. Results of 3-days continuous trawling operations in 1970 suggested that catches might be related to state of tide, better catches being made near high and low water when currents were weaker than when tide was running strongly (Fig. 1). Catch rates decreased over the course of the experiment. The factors governing their behavior are obviously complex but the two main influences appear to be light (and its effect on the fish's prey) and tidal current.

The foregoing data have important implications when considering commercial exploitation of sand

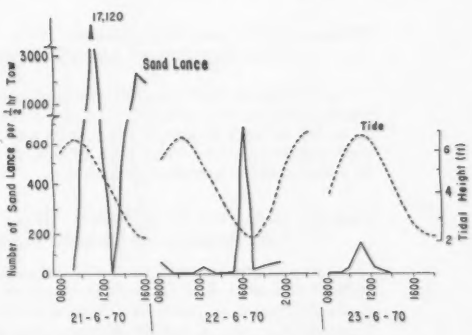

FIG. 1. Changes in numbers of northern sand lance (*Ammodytes dubius*) caught per ½-hr tow (solid line) with tidal heights (broken line) during 3-days fishing on Emerald Bank.

lance on the Nova Scotia Banks. The behavior of the fish makes them available to bottom trawls for very limited periods of time but, unlike the North Sea stocks, they appear to be available, in both summer and winter, to bottom trawling by day and to midwater trawling by night.

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Total Mercury and Methylmercury Content of the American Eel (*Anguilla rostrata*)

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FREEMAN, H. C., AND D. A. HORNE. 1972. The total mercury and methylmercury content of the American eel (*Anguilla rostrata*). J. Fish. Res. Board Can. 30: 454–456.

In American eels (*Anguilla rostrata*) caught in Nova Scotia, the total mercury content was found to be 0.72 ± 0.05 ppm and the methylmercury content was 0.40 ± 0.06 ppm (mean ± SE for 23 fish). Since the mean total mercury content was less than 1 ppm and the toxic methylmercury content was 50% of this, or less than the 0.5 ppm guideline, such eels may be acceptable for human consumption.

FREEMAN, H. C., AND D. A. HORNE. 1972. The total mercury and methylmercury content of the American eel (*Anguilla rostrata*). J. Fish. Res. Board Can. 30: 454–456.

La teneur en mercure total d'anguilles d'Amérique (*Anguilla rostrata*) capturées en Nouvelle-Ecosse est de 0.72 ± 0.05 ppm, celle du méthyl-mercure de 0.40 ± 0.06 ppm (moyenne ± écart type de 23 poissons). Puisque la teneur moyenne en mercure total est inférieure à 1 ppm et que celle du méthyl-mercure toxique est 50% de celle-là, c'est-à-dire inférieure à la concentration tolérée de 0.5 ppm, ces anguilles sont acceptables pour consommation humaine.

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THE eel fishery in the Atlantic provinces of Canada was a small but important fishery with 1,329,000 lb being landed in 1968 (Anon. 1969). Much of this fishery terminated during the past year (1971) when it was found that eels in some locations contained a total mercury content higher than the 0.5 ppm level (wet basis) adopted as the maximum permissible level of mercury in fish for sale in Canada and the United States (Bligh 1970; Berglund et al. 1971). In Sweden only fish containing more than 1.0 ppm of total mercury are banned from the market.

It has been reported that most mercury in fish is in the methylmercury form (Löfroth 1969; Berglund et al. 1971; Uthe 1972). Preliminary analysis of eels in Nova Scotia indicated that the total mercury content was less than 1.0 ppm (wet weight), which would make these eels acceptable for commerce in some European countries. Consequently, both total mercury and methylmercury contents of the American eel (*Anguilla rostrata*) from the Medway River, Nova Scotia, were determined.

Materials and methods — Two groups of eels obtained from the Medway River, Greenfield, Queens County, N.S., were used. The first group (six eels) was caught on the spawning migration to the sea in the fall of 1971; these eels were held in a cage in the Medway River until April 1, 1972 (about 6 months), when they were frozen and stored at –20 C. The second group (17 eels) was caught in the same location in June 1972 and frozen at –20 C after being held alive a few days in a cage near the place of capture.

For mercury determination the eels were thawed, skinned, and homogenates prepared from about 50% of the dorsal muscle, using a blender. Portions of the same homogenate were used for both total mercury and methylmercury determinations.

Total mercury was determined in duplicate on each homogenate by the semiautomated flameless atomic absorption method of Armstrong and Uthe (1971), using a Perkin–Elmer model 403 atomic absorption spectrophotometer equipped with a Perkin–Elmer model 56 recorder. Sampling was performed by a Technicon sampler II and a Technicon proportioning pump.

Methylmercury was determined in duplicate samples according to the semimicro gas–liquid chromatography (GLC) method of Uthe et al. (1972). Samples and

TABLE 1. The total mercury and methylmercury content of American eel (*Anguilla rostrata*) muscle. (Eels 1–6 were held for 6 months in cages suspended in the river; eels 7–23 were freshly caught and held a few days in cages in the river.)

No.	Sex and maturity	Wt (*g*)	MeHg(*as Hg*) (*ppm*)	Total Hg (*ppm*)	$\frac{\text{MeHg}}{\text{Total Hg}} \times 100\%$
1	♀ immature	633	0.59	1.3	46
2	♂ immature	361	1.6	1.3	47
3	♂ mature	131	0.26	0.50	53
4	♂ immature	169	0.37	0.96	39
5	♂ immature	157	0.21	0.46	46
6	♀ mature	163	0.16	0.42	38
7	♀ immature	159	0.19	0.42	45
8	♀ immature	84	0.11	0.57	19
9	♂ mature	319	0.40	0.83	48
10	♂ immature	416	0.14	0.98	14
11	♂ immature	298	0.79	0.98	81
12	♂ immature	236	0.41	0.65	63
13	♀ immature	244	0.52	0.84	62
14	♂ mature	148	0.19	0.80	24
15	♂ mature	161	0.49	0.79	55
16	♂ mature	134	0.36	0.55	65
17	♀ mature	115	0.26	0.40	65
18	♂ mature	129	0.53	0.90	59
19	♂ immature	158	0.56	0.89	63
20	♂ immature	97	0.34	0.76	45
21	♂ immature	88	0.30	0.61	50
22	♀ mature	74	0.17	0.27	62
23	♂ immature	63	0.23	0.42	55
Mean[a]			0.40±0.06	0.72±0.05	50±3

[a]Arithmetic mean ± SE of mean.

standards were injected with Hamilton gastight (No. 1705N) syringes. A Packard model 7831 GLC apparatus, fitted with a ^{3}H-foil electron capture detector and 6-ft × 4-mm glass columns containing 7% carbowax 20 M on chromosorb W 80/100 mesh, treated with dimethyldichlorosilane, was used. The carrier gas (N_2) was maintained at a flow rate of 60 ml/min and the component temperatures were set at 180, 190, 200, and 200 C for the oven, inlet, detector, and outlet, respectively. Responses were recorded on an Electronik 16 Honeywell recorder.

Results and discussion — Mercury values (Table 1) did not indicate any correlation between the levels of mercury and the sex or weights of the eels; this is not unexpected as there was no apparent relation among the weight, sex, or maturity of the fish sampled. The analyses for total mercury showed that the Canadian–U.S. allowable level of 0.5 ppm for marketable fish was exceeded in almost all eels examined; in only two of the 23 eels did the levels exceed 1.0 ppm (both 1.3 ppm).

The level of methylmercury in most of the eels was below 0.5 ppm and averaged 50 ± 3% of total mercury in the eels. This average differed little from that of the group held in captivity (45%) or those frozen a few days after catching (52%), although the range of percentages of methylmercury was much greater in the freshly caught fish (14–81%) than in those held about 6 months (38–53%). The mean methylmercury content of 0.40 ± 0.06 ppm (23 eels) in this study is close to the value of 0.58 ± 0.03 ppm methylmercury (mean 84 eels) calculated from the data of Zitko et al. (1971) for American eels from New Brunswick streams. Bligh (1972) obtained ratios of 0.53 and 0.81 of methylmercury to total mercury for uncontaminated and contaminated American eels, respectively. These values correspond well with those found for the 17 freshly caught eels in the present study.

It has been reported that methylmercury is more toxic than inorganic and other organic mercury compounds and the methyl compound is believed to be the principal causative agent in the neurological diseases at Minamata and Niigata, Japan (Berglund et al. 1971). The 0.5 ppm maximum was established when it was thought that most of the mercury in fish was the toxic methylmercury form. Our results indicate that much of the mercury in the American eel (*A. rostrata*) is in a form other than methylmercury and, since these eels contained less than 1.0 ppm total mercury, it is possible that they are fit for human consumption.

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Age and Growth of the Inconnu (*Stenodus leucichthys*) in Alaska

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ALT, K. T. 1973. Age and growth of the inconnu (*Stenodus leucichthys*) in Alaska. J. Fish. Res. Board Can. 30: 457–459.

Studies of three anadromous groups (Kuskokwim River, lower Yukon River, Kobuk–Selawik area) and two fluvial groups (upper Yukon River and Minto Flats) of inconnu (*Stenodus leucichthys*) showed Kuskokwim River fish to be the fastest growing, reaching 81 cm in 10 years. Fish from the slower-growing Kobuk–Selawik samples attained weights over 20 kg and lengths of 110 cm while fish over 12 kg and 100 cm were uncommon from other waters. Females in all groups studied lived longer than males. Age at sexual maturity ranged from 5 to 9 years for males and 7 to 12 years for females.

ALT, K. T. 1973. Age and growth of the inconnu (*Stenodus leucichthys*) in Alaska. J. Fish. Res. Board Can. 30: 457–459.

L'étude de trois groupes anadromes d'inconnus, *Stenodus leucichthys*, (rivière Kuskokwim, bas Yukon et région Kobuk–Selawik) et de deux populations fluviatiles (haut Yukon et Minto Flats) démontre que les poissons de la rivière Kuskokwim ont le rythme de croissance le plus élevé, atteignant 81 cm en 10 ans. Les individus à croissance plus lente de la région Kobuk–Selawik atteignent un poids de plus de 20 kg et une longueur de 110 cm, alors que les poissons dépassant 12 kg et 100 cm sont rares aux autres endroits. Chez tous les groupes étudiés, les femelles vivent plus longtemps que les mâles. L'âge à la maturité sexuelle varie de 5 à 9 ans chez les mâles, et de 7 à 12 ans chez les femelles.

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THIS note presents new data on age and growth of inconnu (*Stenodus leucichthys*) from the Kuskokwim and lower Yukon rivers, and additional data from Kobuk–Selawik and Minto Flats fish. Objectives were to compare growth of the major Alaskan groups of inconnu and determine longevity and age at sexual maturity.

Limited research had been conducted on the inconnu prior to 1964, when the University of Alaska began a taxonomic and ecological study including age and growth of fish from the upper Yukon River, Chatanika River (Minto Flats), and the Kobuk–Selawik areas (Alt 1969).

Materials and methods — Inconnu were collected from the Holitna, lower Kuskokwim, Koyukuk, lower Yukon, Kobuk, and Selawik rivers and the Minto Flats (Fig. 1). The Koyukuk sample was taken on the spawning grounds in the vicinity of Hughes and 55 miles up the Alatna River. Minto Flats fish were taken in the lower sections of the Chatanika, Tolovana, and Tatalina rivers, which are tributary to the Tanana River. Supporting data include fish from the upper Yukon River tributary streams near the U.S.–Canada border.

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Age and growth data for Holitna River and lower Kuskokwim River fish were combined as they were similar, and tagging results indicated these fish con-

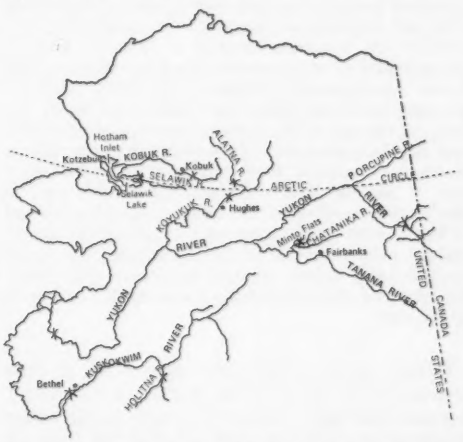

FIG. 1. Sampling groups (X) for the five groups of inconnu (*Stenodus leucichthys*) studied. Map reproduced from U.S.G.S. Alaska Map A.

TABLE 1. Average calculated fork lengths (*mm*) at year-end and sample sizes (in parenthesis) for five groups of Alaskan inconnu (*Stenodus leucichthys*).

Age group	Kuskokwim River (n = 187)	Kobuk–Selawik rivers (n = 547)	Lower Yukon River (n = 204)	Upper Yukon River (n = 66)[a]	Minto Flats (n = 98)
I	188(–)	151(–)	149(–)	126(12)	170(–)
II	326(–)	239(–)	238(–)	215(1)	285(–)
III	417(2)	307(–)	311(–)	285(–)	377(–)
IV	496(3)	369(2)	384(–)	345(11)	466(–)
V	563(5)	430(29)	459(–)	406(15)	534(6)
VI	622(13)	489(49)	530(7)	486(6)	603(20)
VII	676(37)	546(37)	598(11)	548(10)	658(27)
VIII	729(50)	598(43)	668(37)	594(5)	704(21)
IX	777(40)	652(36)	724(48)	644(6)	753(13)
X	810(21)	705(31)	779(58)	–	797(5)
XI	847(11)	759(48)	837(30)	–	828(5)
XII	936(4)	806(60)	896(8)	–	800(1)
XIII	970(1)	855(73)	948(4)	–	–
XIV	–	895(65)	1010(–)	–	–
XV	–	937(26)	1040(1)	–	–
XVI		978(13)	–		
XVII		1016(17)			
XVIII		1072(11)			
XIX		1101(4)			
XX		1094(3)			

[a]Alt 1969.

stitute a single population. They are referred to as "Kuskokwim River inconnu." The Koyukuk and lower Yukon River samples were also combined for the same reason and are termed "lower Yukon River inconnu."

Inconnu were captured by hook and line, drift and set gillnets (2.5-, 3.5-, and 4-inch bar mesh), electrofishing gear, and beach seine. Samples were collected in conjunction with a tagging program so sampling was not representative of various age-classes from different areas. Annuli were identified mainly by the "cutting across" of circuli in the posterior–lateral radius, as circuli laid down at the end of the growing season are incomplete and those laid down at the beginning of the new growing season are complete. Fork length at the end of each year of life was back calculated for all fish used in this study according to the direct proportion method of Van Oosten (1929). Sexes were combined for growth analysis because similar growth rates have been found for male and female inconnu in Alaska (Alt 1969). Maturity was determined through examination of gonads in the field.

Growth Rates — Fish from ages 1 to 20 were represented in the samples (Table 1). Specimens younger than age 3 were taken only in the upper Yukon River. Growth data of older fish from all groups are from a small number of fish and should be interpreted accordingly.

Many of the fish were taken near the spawning grounds or on upstream spawning migrations, accounting for the large number of specimens in the middle and older age groups. This is especially true of the lower Yukon and Selawik–Kobuk samples.

Kuskokwim and Minto Flats fish grow faster than fish of other groups, especially during the first years of life (Fig. 2). Lower Yukon fish show rel-

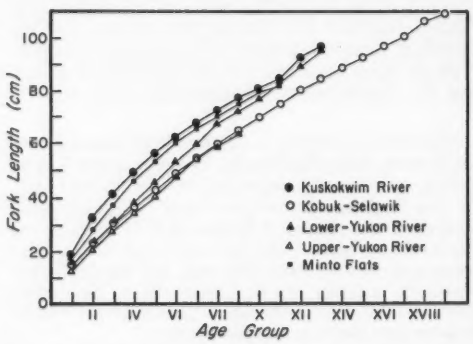

FIG. 2. Growth of five groups of Alaskan inconnu.

atively slow growth up to age 4, then faster growth than other groups studied. Length of lower Yukon River inconnu in the 13th year of life agrees closely with length of Kuskokwim River fish of the same age.

Growth of upper Yukon River fish is slower than for other groups studied.

Inconnu of the Kobuk–Selawik group are slow growing but have a longer life span than other groups and reach the largest size in Alaska (up to 27 kg). These fish have a steady growth rate averaging 52 mm annually between age 3 and 18.

Variation in growth rates of Alaskan inconnu was similar to that of Siberian fish. Fish from the Vilyui River in Siberia reached a length of 118 cm at age 15 (Kirilov 1962) in comparison with 90.4 cm at age 16 for Pechora River fish (Berg 1948).

All Alaskan groups studied showed slower growth rates than fish of the subspecies *Stenodus l. leucichthys* from the basin of the Caspian Sea, which reach 100 cm in 7 years (Podlesnyi 1947).

Minto Flats and Kuskokwim fish grow faster than Great Slave Lake, Northwest Territory, inconnu (Fuller 1955).

Longevity — Maximum ages for inconnu of the five Alaskan groups sampled ranged from 9 to 20.

Upper Yukon River fish have the shortest life span as no fish over age 9 were encountered (Alt 1969). The largest specimen taken was 5.5 kg, but fish of 6.8 kg have been taken by native subsistence fishermen. Maximum age of inconnu sampled from the Minto Flats was 12 years. Kuskokwim River fish sampled reached 13 years of age and a single fish of age 15 was captured in the lower Yukon River. Kobuk–Selawik inconnu have the longest life span of Alaskan fish, often reaching an age of 19 or 20 years.

Females in all groups studied had lived longer than males. Of 273 females taken on the Kobuk River spawning ground, 151 (55%) were age 15 and older, while only 6 males of 447 fish examined were age 15 with the remainder being age 7–14.

Age at Maturity — Ages at initial sexual maturity for Alaskan inconnu are:

	No. in sample	Males	Females
Minto Flats	78	5–7	7–9
Kuskokwim	83	6–8	7–9
Upper Yukon	37	6–7	7–9
Lower Yukon	204	6–9	8–10
Kobuk–Selawik	932	7–9	9–12

Inconnu from the faster-growing Minto Flats and Kuskokwim River groups become sexually mature at an earlier age than the longer-living, slower-growing Kobuk–Selawik fish. Males reach maturity at an earlier age and smaller size than do females.

Acknowledgments — This investigation was conducted with U.S. Federal Aid in Fish Restoration funds.

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A Simple Microscale Vacuum Collector for the Elution of Closely Situated Spots from Thin-Layer Chromatograms

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CHENG, J. Y. 1973. A simple microscale vacuum collector for the elution of closely situated spots from thin-layer chromatograms. J. Fish. Res. Board Can. 30: 460–461.

A "homemade" device, utilizing disposable Pasteur pipettes and a side-armed test tube, is described for convenient quantitative removal and elution of closely separated spots from thin-layer chromatograms. Its simplicity of manipulation and "disposability" after use offer an excellent economic and timesaving elution technique for routine handling of numerous samples.

CHENG, J. Y. 1973. A simple microscale vacuum collector for the elution of closely situated spots from thin-layer chromatograms. J. Fish. Res. Board Can. 30: 460–461.

L'auteur décrit un appareil de "fabrication maison" constitué de pipettes de Pasteur rejetables et d'une éprouvette à tube latéral, qui permet l'enlèvement quantitatif facile et l'élution de taches individuelles rapprochées sur des chromatogrammes en couche mince. Sa manipulation simple et son rejet après usage en font une excellente méthode d'élution, économique et rapide, pour le traitement routinier de plusieurs échantillons.

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THIN-LAYER chromatography has been widely used for studying pigments of marine organisms. The chlorophylls, their degradation products, and some 25–30 carotenoids commonly found from seven classes of marine algae have been separated to varying degrees on thin layers of silica gel (Riley and Wilson 1965), sucrose, polyethylene (Jeffrey 1968), polyamide (Egger and Voigt 1965), alumina-magnesium oxide (Chapman 1965), and magnesium carbonate (Nitsche and Egger 1969) by a variety of solvent systems. Many pigments with similar polarity show closely situated spots on the layers after chromatographic development and require a convenient technical device for microscale collection and elution of such spots. Such a device should preferably be simple to handle, timesaving, and economic in routine handling of many samples, while affording near quantitative recovery of the eluted material. A procedure which utilizes disposable Pasteur pipettes and a test tube with side arm as the main components of a micro-vacuum collector has been devised and proved extremely successful and is in continuous use in this laboratory.

The glass apparatus is composed of four components (Fig. 1), all of which are normally available in a standard laboratory: (A) a 9-inch Pasteur pipette fitted tightly with a glass wool or cotton wool plug; (B) a graduated 2.5-ml centrifuge tube (or a 2-ml volumetric tube) of diameter slightly larger than the broad end of the pipette (C) a test tube (150-mm length, 20-mm outer diameter) with side arm; and (D) a rubber stopper to fit the mouth of the side-armed test tube and with a central hole of diameter sufficiently large to hold the pipette snugly and yet freely after insertion into the tube. In order to prevent movement of the cotton wool plug in the Pasteur pipette, it is advisable to wrap a piece of Whatman No. 1 filter paper around the plugged end of the pipette and then to insert the latter securely into the centrifuge tube. This assembled pipette–centrifuge tube is then housed in the side-armed test tube at the time of use. When the side arm of the apparatus is attached to the vacuum hose (of a water pump), the sample will be aspirated up through the sharp nozzle of the pipette and deposited on the plug. It is good practice to pick up some "blank" adsorbent prior to the sample. This also serves to check whether the whole unit is in good working condition. After the fraction is collected, the filter paper is removed and the adsorbed sample is eluted by one of the following ways: (a) the unit is placed vertically on a suitable test tube rack and another short-capillary Pasteur pipette with sharper tip is inserted into the narrow end of the elution tube, then gentle suction is applied and followed by introduction of small volumes of the appropriate solvent with another pipette (Fig. 2); (b) the unit is laid horizontally, while still under gentle suction, and the solvent is carefully "poured" into the collecting tube from a beaker; or (c) the narrow end of the collecting pipette is cut and the solvent is introduced in any convenient way.

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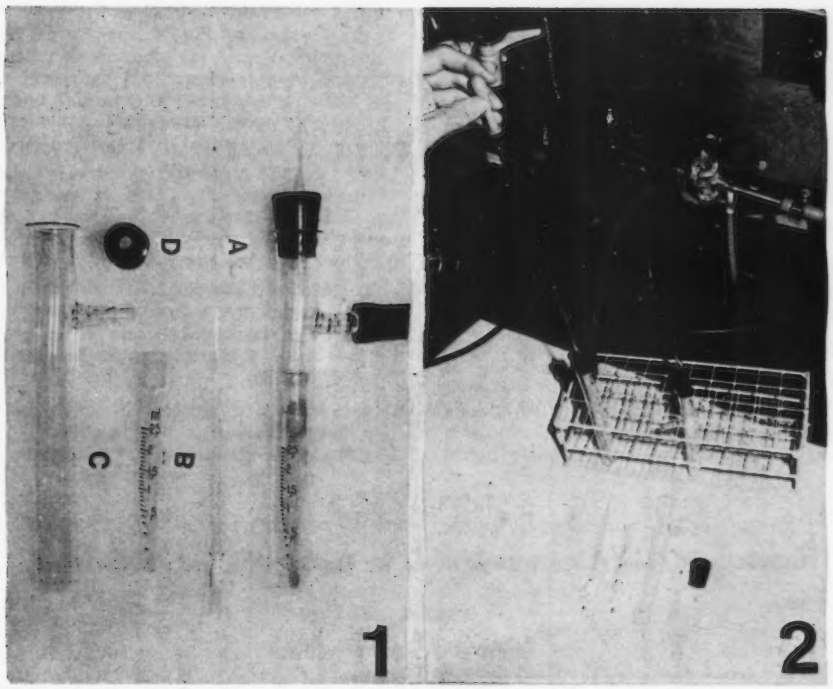

FIG. 1. Micro-vacuum collector. Components A–D are shown as described in the text. The assembled apparatus is shown on the right; FIG. 2. Elution of a collected sample effected with teh apparatus in vertical position.

Cheng — J. Fish. Res. Board Can.

TABLE 1. Quantitative recoveries of some marine algal pigments after application on TLC plates.

Pigment	Source	Amount applied (μg)	TLC adsorbant	Solvent	Extinction coefficients		Spectral[a] recoveries (%)
					λmax. (*nm*)	(*l/g.cm*)	
Chlorophyll *a*	Chroomonas salina[b]	3.0	Sucrose	Acetone	663	92	98
Chlorophyll *c*	Chroomonas salina	9.6	Sucrose	Methanol	635	15.2	94
Alloxanthin	Chroomonas salina	–[c]	Silica gel	Ethanol	453	–	98
α-carotene	Chroomonas salina	3.2	Silica gel	Hexane	445	280	96
Canthaxanthin	A gift from F. Hoffman–La Roche & Co. Ltd.	–[c]	Sucrose	Acetone	469	–	96
Diatoxanthin	Isochrysis galbana	–[c]	Silica gel	Ethanol	452	–	95
Fucoxanthin	Isochrysis galbana	4.6	Sucrose	Ethanol	450	160	95
Peridinin	Amphidinium carteri	4.8	Silica gel	Acetone	466	134	99
Lutein	A gift from Dr B. C. L. Weedon	0.9	Silica gel	Ethanol	447	255	98
Zeaxanthin	A gift from Dr B. C. L. Weedon	–[c]	Silica gel	Ethanol	452	–	96

[a]Means of three determinations (SE ± 2.5%). The measurement of optical density was done on a Unicam SP1800 recording spectrophotometer.

[b]See reference, Cheng et al. 1973.

[c]A concentrated pigment solution, after 100-fold dilution in an appropriate solvent, gives an optical density of 0.3–0.8 at λmax. as indicated in the table. Aliquots of 20 μliter of the sample in each case were applied on the TLC plate.

Tests of recoveries of some marine algal pigments from silica gel G plates have been made to establish the reliability of this elution technique (Table 1). The recoveries of microgram quantities were very satisfactory and the capacity to collect adsorbent from thin-layer plates could be conveniently extended up to 600-mg silica gel covering an area of 15 cm × 1 cm on a plate of 0.5-mm thickness. The feasibility of collecting very closely situated spots without the risk of cross-contamination is due to the sharp tip of the Pasteur pipette used, and the low cost as well as disposability of the latter after use make the apparatus both economic and timesaving. The "micro-vacuum cleaner" of Riley and Wilson (1965) is similarly simple but not as timesaving. The special elution tube designed by Jeffrey (1968), the adaptation of Swinny filter holders reported by Nagel and Dittmer (1969), and the device of Sudilovsky and Hinderaker (1972) are expected to perform well but they are expensive and require cleaning after each use.

In conclusion, this "homemade" micro-vacuum collector offers the advantages of simplicity, economy, and timesaving, and is particularly recommended to those who must routinely handle a large number of chromatograms.

Acknowledgments — The encouragement and support of Dr N. J. Antia are deeply appreciated. Thanks also to Dr S. W. Jeffery for her valuable suggestions and critical review of the manuscript, and to Dr B. C. L. Weedon and F. Hoffman–La Roche & Co. Ltd. for supplying the authentic carotenoid specimens.

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Preparation and Properties of 3-Trifluoromethyl-4-Aminophenol

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LECH, J. J. 1973. Preparation and properties of 3-trifluoromethyl-4-aminophenol. J. Fish. Res. Board Can. 30: 461–463.

This note describes a method for the preparation of 3-trifluoromethyl-4-aminophenol (RTFM), a metabolite of 3-trifluoromethyl-4-nitrophenol (TFM), and some of its physicalchemical properties, including infrared spectrum, *pKa*, and gas chromatography.

LECH, J. J. 1973. Preparation and properties of 3-trifluoromethyl-4-aminophenol. J. Fish. Res. Board Can. 30: 461–463.

Nous décrivons une méthode pour la préparation chimique de 3-trifluorométhyl-4-aminophénol (RTFM), un métabolite de 3-trifluorométhyl-4-nitrophénol (TFM), et quelques-unes de ses propriétés physicochimiques, y compris spectre infrarouge, *pKa*, et chromatographie en phase gazeuse.

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3-TRIFLUOROMETHYL-4-NITROPHENOL (TFM) is a selective larvicidal agent which is currently being used to control the population of the sea lamprey (*Petromyzon marinus*) in the upper Great Lakes. Recent studies have indicated that TFM is metabolized to 3-trifluoromethyl-4-aminophenol (RTFM) in the rat (Lech 1971) and in anerobic lake sediment incubations (Bothwell et al. 1972). In the course of these biotransformation studies it was necessary to utilize synthetic RTFM as a standard in the metabolic studies. This report is concerned with the preparation of RTFM and some of its properties that may aid in the design of analytical procedures for metabolite residues.

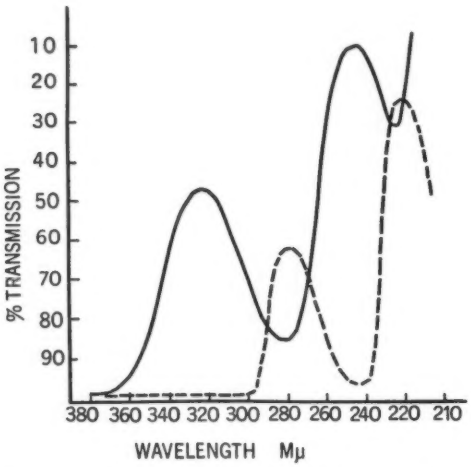

FIG. 1 Ultraviolet spectrum of 3-trifluoromethyl-4-aminophenol. Solid curve in 0.1-N NaOH. Broken curve in 0.1-N HCl.

Materials and methods of TFM — TFM was obtained from Mr J. Howell, U.S. Department of the Interior Lamprey Research Laboratory, Hammond Bay, Michigan. Elemental analysis was performed by Galbraith Labs, Knoxville, Tennessee. The QF-1 gas-chromatographic column was obtained from Applied Science Labs, State College, Pennsylvania.

3-Trifluoromethyl-4-aminophenol was prepared from 3-trifluoromethyl-4-nitrophenol by reduction with hydrogen according to the following procedure: 40 g of crystalline 3-trifluoromethyl-4-nitrophenol, mp 74–75, was dissolved in 300 ml of absolute ethanol, and 200 mg of 5% palladium on charcoal (Girdler G81C) was added to the ethanolic solution. Reduction was carried out in a Parr apparatus at a pressure of 60 psi H_2. When the reaction was complete, the mixture was filtered to remove the catalyst, the filtrate was placed in an ice bath at 0 C, and HCl gas was bubbled through the mixture until precipitation ceased. The white precipitate

TABLE 1. Molar extinction coefficients and absorption maxima of 3-trifluoromethyl-4-aminophenol (RTFM–HCl).

Conditions	Absorption maximum (*nm*)	Molar extinction (ϵ)
0.1-N NaOH	326	3.40×10^3
	248	1.02×10^4
0.1-N HCl	277	1.95×10^3
	235	5.85×10^3
Distilled H_2O	308	2.95×10^3
	235	8.50×10^3

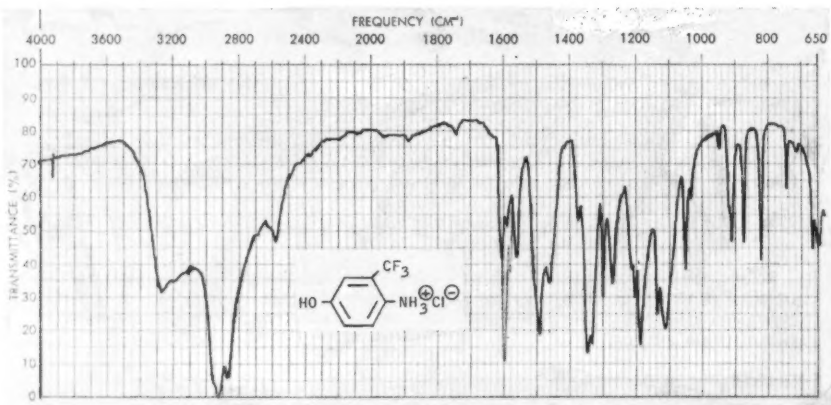

FIG. 2 Infrared spectrum of 3-trifluoromethyl-4-aminophenol. HCl in mineral oil mull.

TABLE 2. Thin layer chromatography of 3-trifluoromethyl-4-aminophenol on silica gel.

Solvent system			rf
Benzene 75	Acetone 25	NH_4OH 0.5	0.65
Benzene 50	Ether 25	Glacial acetic acid 0.5	0.50

was then collected by vacuum filtration and was washed several times with ice-cold acetone. The resulting material was dried under vacuum and stored under nitrogen. The yield was 27 g of white fluffy powder which decomposed at 210–220 C. Elemental analysis indicated that this material contained 39.19% C, 26.95% F, and 3.28% H. The calculated values were 39.32% C, 26.69% F, and 3.31% H.

Thin-layer chromatography was performed on glass plates precoated with silica gel containing a fluorescent indicator. Solvent systems consisted of benzene: acetone: NH_4OH, 75:25:0.5 (Solvent I) and benzene: diethyl-ether: glacial acetic acid, 50:25:0.5 (Solvent II). After development (30 min), the plates were either visualized under ultraviolet light or sprayed with 0.5% *p*-dimethyl-aminobenzaldehyde in 0.1-N HCl in absolute ethanol. With the latter method, primary aromatic amines appear as yellow spots on a white background.

The ultraviolet spectrum of 3-trifluoromethyl-4-aminophenol was determined in aqueous solution utilizing a recording Beckman DB spectrophotometer. Aqueous solutions were prepared using the hydrochloride salt and *p*H adjustments were made with 5-N NaOH.

The infrared spectrum of 3-trifluoromethyl-4-aminophenol HCl was run in a Perkin Elmer Model 700 spectrophotometer in a mineral oil mull.

The *pKa* of the amine and phenolic group of 3-trifluoromethyl-4-aminophenol was determined spectrophotometrically essentially as described by Jaffé and Orchin (1964).

Samples of 3-trifluoromethyl-4-aminophenol were prepared for gas chromatographic analysis by acetylation with 10% acetic anhydride in benzene. The reaction was carried out in sealed glass ampules at 50 C for 30 min, after which the product was chromatographed on a Beckman GC-4 gas chromatograph utilizing a nonradioactive electron capture detector. The column used was a 6-ft × 4-mm glass column packed with 10% QF-1 on 80/100 mesh Gas Chrom Q. The column temperature was 170 C and that of the detector was 200 C.

Results — The ultraviolet spectrum of RTFM is shown in Fig. 1. The solid line shows the spectrum in 0.1-N NaOH and the dashed line in 0.1-N HCl. The molar extinction coefficients and absorption maxima are shown in Table 1. Figure 2 shows the infrared spectrum of RTFM·HCl. The lightly shaded peak at about 1600 cm^{-1} is the 1601 cm^{-1} calibration peak of polystyrene.

Using the conditions described in the materials and methods section, the retention time of acetylated RTFM was found to be 5.8 min. Table 2 shows the mobility of RTFM in two solvent systems using silica gel thin-layer chromatography plates.

The *pKa* of the amino group was found to be 2.76 and that of the phenolic group was 10.25.

Acknowledgments — The author wishes to thank Mr J. Allen of the Bureau of Sport Fisheries and Wildlife, La Crosse, Wis., and Dr J. Friedman of Lakeside Laboratories, Milwaukee, Wis., for their aid in this study.

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Uteriporus vulgaris Bergendal, 1890, A Marine Triclad New to North America[1]

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TOMKIEWICZ, W., AND I. R. BALL. 1973. *Uteriporus vulgaris* Bergendal, 1890, a marine triclad new to North America. J. Fish. Res. Board Can. 30: 464–466.

Uteriporus vulgaris Bergendal, 1890, a marine triclad of the family Uteriporidae, is recorded from New Brunswick and the Gaspé coast, Quebec. These are the first records of this species, genus, and family in North America. A brief description of the material is given.

TOMKIEWICZ, W., AND I. R. BALL. 1973. *Uteriporus vulgaris* Bergendal, 1890, a marine triclad new to North America. J. Fish. Res. Board Can. 30: 464–466.

Les auteurs signalent la présence d'*Uteriporus vulgaris* Bergendal, 1890, triclade marin de la famille des Uteriporidae, près des côtes du Nouveau-Brunswick et de Gaspé, Québec. Il s'agit de premières mentions de l'espèce, du genre et de la famille en Amérique du Nord. Ils donnent une brève description du matérial.

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UTERIPORUS VULGARIS is a marine triclad widely distributed along the northwestern coast of Europe. Bergendal (1890) first described this worm, which he collected from the west coast of Sweden, and added to the description later (Bergendal 1892, 1896). Further descriptions are provided in the monographic studies of Böhmig (1906) and Wilhelmi (1909). Subsequent to its original discovery, *U. vulgaris* has been recorded from northern Norway and the White Sea (see Wilhelmi 1911), the coast of Ireland (Southern 1936), the German coast of the Baltic Sea (Ax 1960), the Netherlands (Hartog 1963), northern France (Stephan-Dubois and Masson 1965), and Plymouth, U.K. (Hartog 1968). Hartog (1963) has provided the most detailed account of its ecology.

As a result of independent surveys carried out in the Bay of Fundy, New Brunswick (WT), and the Gaspé coast, Quebec (IRB), we are now able to report, for the first time, the occurrence of this genus and species in North America. Hitherto, the family Uteriporidae has not been recorded unequivocally in North America. The transference of the Probursidae Hyman, 1944, to the Uteriporidae by de Beauchamp (1961) seems unwarranted, although it has been followed by Gosner (1971).

The species was not recognized in the field by either of us. In New Brunswick its superficial similarity to *Foviella affinis* (Oersted) Bock, 1925, with which it coexists, made identification difficult. Its identity was confirmed, again independently by both of us, from serial sections and by subsequent comparison with Bergendal's original material in the Naturhistoriska Riksmuseet, Stockholm.

The following brief description is provided to facilitate recognition of the species by North American workers:

Uteriporus vulgaris Bergendal, 1890

Synonymy in Wilhelmi (1909, 1911)

External features (*Fig. 1A*) — Small slender species, up to 7 mm in length. With rounded anterior end, and two eyes situated close together and distant from the anterior margin. Pharynx about one-quarter of the body length, its root just over halfway along the body. With two gonopores, a bursal pore immediately behind the pharyngeal pore, and posterior to this a common

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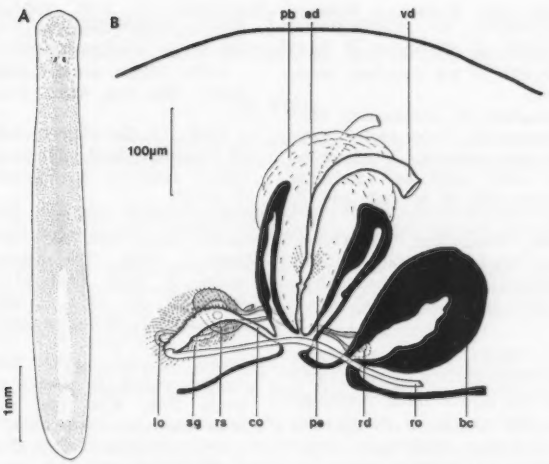

FIG. 1. (A) *Uteriporus vulgaris*: External features drawn from a photograph of a living animal from Ste. Anne des Monts, Que.; (B) *U. vulgaris*: Diagrammatic sagittal section of the copulatory apparatus, viewed from the right side. Specimen from Cap Renard, Que. (bc, bursa copulatrix; co, common ovovitelline duct; ed, ejaculatory duct; lo. left ovovitelline duct; pb, penis bulb; pe, penis papilla; rd, receptacular duct; ro, right ovovitelline duct; rs, accessory seminal receptacle of the receptacular duct; sg, shell glands; vd, vas deferens) For clarity the right receptacular duct has been omitted from the drawing.

gonopore. Dorsal surface variable in color; milky-white, yellowish-orange, or pale brown. In the more heavily pigmented forms the regions above the pharynx and copulatory organs are always paler. Ventral surface without pigment.

Reproductive organs (*Fig. 1B*) — There are two genital pores: the bursa copulatrix, which is just behind the pharynx, opens anteriorly to, and independently of, the common gonopore. These are the characteristics of the family Uteriporidae.

The male atrium and the penis are located posteriorly to the bursa. The penis consists of a muscular bulbar region and a large, free, conical, and muscular papilla which is ventro-caudally directed. Lateral to the pharynx are the large, convoluted, vasa deferentia, which bend upwards in the region of the penis, make a medial bend, and enter the penis bulb. They then extend distally and unite to form the ejaculatory duct, which is about one-half the length of the penis. The testes are regularly arranged between the intestinal diverticula, are principally ventral in position, and extend from the region of the brain to the posterior tip of the animal. There are 7–12 pairs of follicles.

The two ovaries are small and located medially behind the brain. There are two ovovitelline ducts, one from each ovary, and these proceed posteriorly to the region behind the male copulatory apparatus, where they bend medially and unite to form the common ovovitelline duct. This duct, into which open extensive shell glands, travels anteriorly and enters the ventro-posterior wall of the male atrium, just above the common gonopore. It is also connected to the bursal canal by two small ducts, the receptacular ducts. These branch from the sides of the bursal canal, pass posteriorly along the sides of the copulatory apparatus, and connect with the proximal part of the common ovovitelline duct. Near to their junctions with the common ovovitelline duct, each receptacular duct is enlarged to form an accessory seminal receptacle.

Identification — With experience *U. vulgaris* may be distinguished from the superficially similar *F. affinis* by its more slender appearance, lighter color, and by the smoother curvature of the anterior margin. In *F. affinis* the head tends to a triangular shape. The presence of two genital pores and a well developed bursa copulatrix are the principal internal features that may be used to distinguish *U. vulgaris* from *F. affinis*. *U. vulgaris* is also similar to some lightly pigmented and white species of *Procerodes*, but thus far such species are not known from eastern North America.

Occurrence — Ste Anne des Monts, Gaspé Peninsula, Quebec. I. R. Ball, June 24, 1971. Under Rocks in the

intertidal zone. Coexisting with *Procerodes littoralis* and *Monocelis* spp.

Cap Renard, Gaspé Peninsula, Quebec. I. R. Ball, June 25, 1971. Under Rocks in the intertidal zone. Coexisting with *P. littoralis*.

St Andrews, New Brunswick. W. Tomkiewicz, September 4, 1971, and subsequently. Under rocks in the intertidal zone. The rocks were embedded in a reddish-orange "clay" substrate, with which the specimens tended to blend. Coexisting with *P. littoralis* and *F. affinis*.

In the Naturhistoriska Riksmuseet, Stockholm, there is a set of sections of this species, which are of a specimen apparently collected from St John's, Newfoundland, on June 13, 1911, by O. Hentschel.

On Gaspé coast *U. vulgaris* is not common, and all specimens are infested heavily with gregarine parasites. From the above data, however, it seems probable that the species is widely distributed on the Atlantic coast of Canada from Newfoundland to the Bay of Fundy.

Acknowledgments — The junior author wishes to thank sincerely Prof. T. G. Karling for the support and facilities which he made available during a visit to the Naturhistoriska Riksmuseet, Stockholm, Sweden.

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Changes in Hematocrit and Hemoglobin in Atlantic Salmon (*Salmo salar*) as a Result of Furunculosis Disease

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FODA, A. 1973. Changes in hematocrit and hemoglobin in Atlantic salmon (*Salmo salar*) as a result of furunculosis disease. J. Fish. Res. Board Can. 30: 467–468.

Decreases in the hematocrit and hemoglobin levels in hatchery-reared juvenile Atlantic salmon (*Salmo salar*) were associated with a severe infection of *Aeromonas salmonicida*, the causative agent of furunculosis disease. The lowering of blood hematocrit and hemoglobin levels were attributable to hemorrhages resulting from the infection.

FODA, A. 1973. Changes in hematocrit and hemoglobin in Atlantic salmon (*Salmo salar*) as a result of furunculosis disease. J. Fish. Res. Board Can. 30: 467–468.

L'auteur démontre une association entre la réduction des teneurs de l'hémoglobine et de l'hématocrite du sang chez des saumoneaux de pisciculture (*Salmo salar*) et une infection sévère causée par *Aeromonas salmonicida*, agent responsable de la furonculose des salmonidés. La diminution des valeurs de l'hématocrite et de l'hémoglobine est attribuable aux hémorragies qui résultent de l'infection.

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THIS report records the influence of *Aeromonas salmonicida*, the causative agent of furunculosis disease, on hematocrit and hemoglobin values in juvenile Atlantic salmon (*Salmo salar*). According to McGraw (1952), furunculosis is one of the most destructive diseases of hatchery fish. In Canada, furunculosis has been recorded from British Columbia in the Elk River and in a salmon rearing pond at Cultus Lake (Duff and Stewart 1933). The first major outbreak of furunculosis in Eastern Canada was at a Quebec trout hatchery in 1952 (Margolis 1954).

During the period September 27–October 8, 1971, the mortality in one of the yearling ponds at Mactaquac Fish Culture Station, New Brunswick, increased to approximately 1.2% per day from an average of about 0.027% per day. In an attempt to determine the cause of the abnormally high rate of mortality, samples of both sexes were examined for external and internal lesions, and blood samples were taken for determination of hematocrit and hemoglobin.

Materials and methods — Three samples of eight fish each, representing apparently normal fish, moribund fish, and fish that had died very recently, were examined. Prior to blood sampling, live fish were anesthetized with MS 222 (0.1 g/liter) at a dose sufficient to produce complete loss of equilibrium in less than 1 min (Black and Conner 1964). The blood sample was drawn from the dorsal aorta in the region of the caudal peduncle posterior to the anal fin by means of a 1-ml syringe coated with a dry film of heparin (1000 I.U./ml). Blood mixed with anticoagulant was taken up in duplicate standard capillary tubes and centrifuged for 3 min at 11,000 rpm. Microhematocrit values were obtained using an IEC reader. Hemoglobin levels were measured using the cyanomethemoglobin method according to Crosby, Mann, and Furth (Hawk et al. 1965).

External hemorrhages in the ventral areas were evident in both dead and moribund fish. The lesions were most prominent around the bases of the pectoral and pelvic fins. In some fish these lesions were located subcutaneously, particularly in the area between the pectoral fins. This is similar to Herman's (1968) observation of hemorrhage at the bases of the fins, particularly the pectorals, generally accompanying furunculosis infection. Histopathological examination also revealed massive hemorrhage in abdominal muscle and visceral organs of the fish due to bacterial infection. The bacterium was isolated and identified as *Aeromonas salmonicida*, the causative agent of furunculosis, by C. Frantsi, Fish Disease Biologist at Mactaquac Fish Culture Station.

Results and discussion — In fish that had recently died the hemoglobin level had dropped from a normal value of 9.5 g/100-ml blood to 2.7 g/100-ml blood (Table 1). Hematocrit was reduced to an average of 11% and ranged from 5 to 15%. Hematocrit was very low compared with that of healthy fish, which ranged from 35 to 50%. In moribund fish, hematocrit and hemoglobin were less than 50% of the value in normal fish. Lower hemoglobin

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TABLE 1. Mean hematocrit and hemoglobin values and, in parenthesis, ranges (values for eight fish) for healthy and furunculosis infected Atlantic salmon (*Salmo salar*).

Condition of fish	Weight (*g*)	Fork length (*cm*)	Hematocrit (%)	Hemoglobin (*g/100 ml of blood*)
"Normal"	36.7±7.69[a]	15.1±0.98	44.5±3.5	9.5±0.86
	(30.7–49.7)	(14.3–17.2)	(39–50)	(8.64–11.16)
Moribund	32.0±7.16	14.5±1.1	18.8±2.89	4.3±0.39
	(18.9–44.1)	(12.3–16.2)	(16–25)	(3.72–5.04)
Recently died	34.7±5.18	14.9±0.5	11.0±3.5	2.7±0.73
	(28.7–44.1)	(14.2–16.5)	(5–15)	(1.5–3.8)

[a]One standard deviation of mean.

and hematocrit values in dead than in moribund specimens was probably associated with the degree of progression of the disease. Sharp decline in their values to the extent indicated here would result in diminished oxygen transport capabilities and probably cause suffocation of fish.

Clear plasma obtained after centrifugation of blood indicated hemalysis had not occurred in situ. Therefore, the decline in both hematocrit and hemoglobin value was not due to the destruction of red blood cells. Low values of hemoglobin and hematocrit were likely related to severe internal and external hemorrhages. Blood in examined infected fish appeared to be of low viscosity and the sedimentation rate of blood cells was very rapid. This observation is in agreement with Schumacher et al. (1956), who recorded faster sedimentation rates in blood from furunculosis infected brook trout than from normal fish.

Acknowledgments—I am obligated to Messrs D. B. Lister, T. G. Carey, and C. L. Dominy for reviewing the manuscript. I am also grateful to Dr C. Frantsi for conducting the histopathological examinations.

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Additional Notes on Polychaetes as Intermediate Hosts of Helminth Parasites of Vertebrates

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MARGOLIS, L. 1973. Additional notes on polychaetes as intermediate hosts of helminth parasites of vertebrates. J. Fish. Res. Board Can. 30: 469–470.

Additions and corrections to the records of larval helminths in polychaetes reviewed by Margolis (1971) are summarized.

MARGOLIS, L. 1973. Additional notes on polychaetes as intermediate hosts of helminth parasites of vertebrates. J. Fish. Res. Board Can. 30: 469–470.

L'auteur présente un résumé des additions et corrections aux signalements d'helminthes larvaires chez les polychètes passés en revue par Margolis (1971).

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THE role of polychaetes in the life cycle of helminth parasites of vertebrates was reviewed recently (Margolis 1971). In a table listing the trematodes utilizing polychaetes as second intermediate hosts, certain reports of *Gymnophallus choledochus* Odhner 1900 were inadvertently omitted. The main purpose of this communication is to summarize these additional records and review some new information, most of which bears on the previously summarized records.

Metacercariae of *G. choledochus* have been found in *Nereis diversicolor* collected at the mouth of the Orne River on the English Channel coast of France (Prévot 1966), in *N. diversicolor* and *Nephthys* [*Nephtys*][1] *caeca* in Welsh waters (Bowers and James 1967), and in *N. diversicolor*, *Nephthys* [*Nephtys*] *hombergi*, and *Arenicola marina* from the North Sea (Loos-Frank 1969). Prévot (1966) left the matter of the identity of his specimens undecided, considering them to be either *G. choledochus* or *G. deliciosus* (Olsson 1893) Odhner 1900. Bowers and James (1967) implied that their specimens were conspecific with those reported by Prévot (1966) and suggested that they probably belong to *G. deliciosus*. Loos-Frank (1969, 1971), however, regarded Prévot's (1966) and Bowers and James' (1967) material as *G. choledochus*.

Also of relevance to the discussion of gymnophallids in polychaetes is Bowers and James' (1967) relegation of *Gymnophallus nereicola* Rebecq and Prévot 1962, whose metacercarial stage occurs in *N. diversicolor* on the Mediterranean coast of France (see Table 1, Margolis 1971), to the synonymy of *Meiogymnophallus minutus* (Cobbold 1859) Bowers and James 1967. Loos-Frank (1971) and Bartoli (1972), however, retained it as a separate species under its original name. Bartoli (1972) considered *N. diversicolor* to be the only intermediate host of *G. nereicola*.

Shortly after the publication of my review, Reimer et al.'s (1971) paper on larval helminths in North Sea zooplankton appeared. In it, *Opechona bacillaris* (Molin 1859) Looss 1907, *Monilicaecum* sp. (Didymozoidae larva), and *Contracaecum* sp. were reported from *Tomopteris helgolandica*. Based on earlier correspondence with Dr Reimer I concluded (Margolis 1971), apparently incorrectly, that these records were from the Baltic Sea.

Stunkard (1972), working at Woods Hole, established additional experimental second intermediate hosts of *Lepocreadium setiferoides* (Miller and Northup 1926) Martin 1938, among them the spionid polychaetes *Polydora ciliata* and *P. ligni*. The following polychaetes were refractory to experimental infection by *L. setiferoides* cercariae: *Aphrodita hastata*, *Arabella iricolor*, *Clymenella torquata*, *Glycera americana*, *G. dibranchiata*, *Lepidonotus squamatus*, *Nephtys bucera*, *Notomastus latericeus*, *Pectinaria* [*Cistenides*] *gouldii*, *Scoloplos* [*Haploscoloplos*] *fragilis*, and *Sthenelais boa*. The natural second intermediate hosts of *L. setiferoides* apparently remain unknown.

Leonereis [*Laeonereis*] *culveri* was reported by Vernberg and Vernberg (1971) as a natural second intermediate host of *Zoogonus lasius* (Leidy 1891) Stunkard 1940 in the Beaufort, North Carolina

[1]Names in brackets throughout the paper are those used by Hartman (1959, 1965), whose polychaete nomenclature was followed in my original review.

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region. Previously, *Nereis virens* had been found to be the second intermediate host in the Woods Hole region. The work of Vernberg and Vernberg is of special interest since it represents the first published account of the effects of larval helminths on their polychaete hosts. Using experimentally infected *L. culveri*, they demonstrated that the thermal metabolic response and cytochrome *c* oxidase activity of the polychaete are modified by *Z. lasius* metacercarial infection. In addition, they stated that "massive" invasion by *Z. lasius* cercariae is lethal for *L. culveri*.

Additional information on echinostomatid metacercariae in *N. diversicolor* was published by Reimer (1971) for the central Baltic Sea area. Metacercariae of *Himasthla secunda* (Nicoll 1906) Dietz 1909 were produced experimentally in this polychaete and natural infections with an undetermined species of Himasthlinae were observed.

Of the four reports of cestode larvae in polychaetes (Margolis 1971), one apparently is erroneous. According to Dr R. Ph. Dollfus (personal communication), A. de St. Joseph's *Tetrarhynchus* larva, which he re-examined many years ago, is in fact an arthropod larva!

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Mercury, DDT, and PCB in Harbour Seals (*Phoca vitulina*) from the Bay of Fundy and Gulf of Maine

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Samples of blubber, longissimus muscle, liver, and cerebrum from 12 harbour seals (*Phoca vitulina*) were analyzed for DDT, dieldrin, PCBs, and total mercury content. The results were compared with those obtained previously for harbour porpoises (*Phocoena phocoena*). DDT and PCB levels appear to be of the same magnitude in the fat of seals from both southern New Brunswick and southern Maine, being lowest in a lactating female. Virtually no *o,p'*-DDT and relatively little dieldrin were found in seal fat, in contrast to porpoises, which contained significant amounts of both in the depot fat. Mercury levels were generally similar to those found for porpoises, but total liver Hg was considerably greater in adults from the New Brunswick islands than in those from the southern Maine ledges.

GASKIN, D. E., R. FRANK, M. HOLDRINET, K. ISHIDA, C. J. WALTON, AND M. SMITH. 1973. Mercury, DDT, and PCB in harbour seals (*Phoca vitulina*) from the Bay of Fundy and Gulf of Maine. J. Fish. Res. Board Can. 30: 471–475.

Les auteurs ont effectué des dosages de DDT, dieldrine, PCB et mercure total sur la graisse, le muscle longissimus, le foie et le cerebrum de 12 phoques communs (*Phoca vitulina*). Ils comparent ces résultats avec ceux d'analyses antérieures sur le marsouin commun (*Phocoena phocoena*). Les niveaux de DDT et de PCB sont semblables chez les phoques du sud du Nouveau-Brunswick et du sud du Maine, et sont le plus bas chez une femelle en lactation. La graisse des phoques ne contient pratiquement pas de *o,p'*-DDT et relativement peu de dieldrine, contrairement aux marsouins où les deux sont présents en quantité appréciables dans les lipides de réserve. En général, les niveaux de mercure chez les phoques sont semblables à ceux des marsouins, mais la teneur en mercure total du foie est beaucoup plus élevée chez les adultes provenant des îles du Nouveau-Brunswick que chez ceux capturés sur les chaînes de rochers du sud du Maine.

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HIGH organochlorine pesticide and mercury levels have recently been reported from harbour porpoises (*Phocoena phocoena*) in the Bay of Fundy region (Gaskin et al. 1971, 1972). During 1971 sampling for these contaminants in harbour seals (*Phoca vitulina*) was carried out in the mouth of the Bay of Fundy and in the adjacent Gulf of Maine.

The harbour seal is a widespread open water species frequently found in estuaries and rivers, as well as around coastal ledges. A Fisheries Department bounty system has encouraged hunting. The species is probably now not nearly as common in the Bay of Fundy as in previous years. However, it is in no danger of extinction, and some scores of animals can be found each year in prime feeding areas. The two most common items of diet in this region are herring and winter flounder (Mansfield 1967), although the species is well known as an opportunistic feeder and occupies an ecological niche close to that of the harbour porpoise. There-

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fore, the purpose of this study was to use available specimens to compare the pesticide and heavy metal residues in the two species. The senior author and others are conducting an ecological study of the harbour porpoise and other marine mammals in the Bay of Fundy and adjacent areas. As part of this program, the distribution of contaminants of potential importance is being assessed by analyses of selected target organs from animals of known sex, size, and reproductive condition.

Materials and methods — Six harbour seals were collected from offshore ledges near Boothbay Harbour, southern Maine; five from ledges off the northern end of Grand Manan Island; and one from Cummings Cove on the northwestern coast of Deer Island, New Brunswick, in the period June–September 1971. Tissue samples were chilled to about 10 C and frozen to –20 C as soon as field conditions permitted (2–12 hr). Analysis for total mercury was as previously described by Gaskin et al. (1972). Elemental mercury is released and read as a cold vapour at 253.7 nm with an AA-5 Techtron Atomic Absorption Spectrophotometer. For organochlorine compound estimation macerated representative samples were subjected to exhaustive soxhlet fat extraction with hexane, and 1 g of extracted fat of each was cleaned on a 5% deactivated florisil column according to the method described by Langlois et al. (1964). Chlorinated hydrocarbons were eluted from the column with 20% dichloromethane in hexane. After a preliminary gas chromatographic run to assess residue levels of DDTs and PCBs, a suitable aliquot dissolved in acetone was introduced to an activated coconut charcoal column to separate the DDT group from PCBs present, following a modification of the method originally described by Berg et al. (1972). The DDT group was eluted first with 25% acetone in ether, PCBs were next removed from the column with benzene. Both fractions were passed through a mixed phase column for residue qualification and quantitation by gas chromatography. The following recovery rates were obtained for extraction and cleanup of chlorinated hydrocarbons in the seal tissue: *p,p'*DDE, 97.8%; *o,p'*DDT, 91.2%; *p,p'*DDD, 94.6%; *p,p'*DDT, 86.6%; dieldrin, 89.3%; aroclar 1254, 91.2%; aroclar 1260, 90.8%. Corresponding recovery data for the compounds after separation by the charcoal column were as follows. In the fraction containing DDTs: *p,p'*DDE, 93.5%; *o,p'*DDT, 96.9%; *p,p'*DDD, 95.2%; *p,p'*DDT, 97.7%; dieldrin, 96.2%. In the fraction containing PCBs; aroclar 1254, 89.2%; aroclar 1260, 90.1%. As is customary in the literature, the tabulated results are presented as obtained, and not corrected for recovery percentages. In the previous study on organochlorine residues in porpoises, PCBs were not accurately measured, but instead compared approximately with peaks for DDE.

Results and discussion — Results obtained are presented in Tables 1 and 2. Within the limits of the sample size it appears that the contamination levels of both DDT and PCBs are of similar magnitude in harbour seal depot fat in both areas. Of special note is the relatively low level of DDT and PCBs in specimen #12 (8.64 ppm and 7.10 ppm, respectively). This was a lactating female. Gaskin et al. (1971) found that, in the Bay of Fundy region, organochlorine residue levels were much lower in lactating harbour porpoises than in immature or resting females.

In the organs examined the patterns of organochlorine residue distribution parallel in a general way those found in harbour porpoises in the same area. However, there are some important differences. While $\sum$ DDT levels per gram of extractable fat were generally less than half those recorded for porpoises, residues of *o,p'*-DDT were quite insignificant in seal fat. In porpoises, on the other hand, average levels of this compound ranged from a low of 2.41 ppm in pregnant or lactating females or both to a high of 17.84 ppm in weaned and adult males. Similarly, dieldrin levels in seal fat were very low in both areas, with a maximum of 0.38 ppm in specimen #3 (blubber sample, 94.5% fat). The maximum for harbour porpoise was 13.1 ppm in a 1969 specimen (blubber sample 98% fat).

Contamination levels of both DDT and PCBs in adults and juveniles appear to be greater than those reported for seals taken on the coast of Scotland (Holden and Marsden 1967) and the Gulf of Finland (Jensen et al. 1969). However, the authors are aware that comparable or higher levels have been cited for grey seals (*Halichoerus grypus*) from the Canadian Atlantic coast and European waters in several unpublished manuscripts (ICES mimeographed reports 1969–71).

Whether the present results show individual mercury equilibrium levels is not known. However, total mercury in muscle and cerebrum, but perhaps not in liver, is probably close to that which might be anticipated in a species at the trophic level of the harbour seal. Total liver mercury is much greater in the adults from the islands of the mouth of the Bay of Fundy than in those from the coast of southern Maine. A limited number of analyses (liver only) were made of the amount of methylmercury present in the total (see final columns of Tables 1 and 2). The percentage of methylmercury was lowest in the two specimens with highest total mercury (#11, 12), 0.30 ppm of 13.10 ppm, and 0.93 ppm of 50.90 ppm, respectively. Liver methylmercury varied from a low of 0.18 ppm in 1.72 ppm (#7) to a high of 1.01 ppm in 7.90 ppm (#2). In harbour porpoises, Gaskin et al. (1972) noted that, as specimens with progressively higher total liver mercury levels were examined, the percentage of methylmercury decreased. If the actual level of

TABLE 1. DDT, PCB, and total Hg in harbour seals (*Phoca vitulina*) from Boothbay Harbour, Maine, in 1971. All results expressed as parts per million of thawed tissue as received. N.D. = "not detected."

Specimen no.	Date taken	Sex	Length (*cm*)	Weight (*kg*)	Tissue	% fat in tissue	*p,p'*-DDE	*p,p'*-DDD	*o,p'*-DDT	*p,p'*-DDT	ΣDDT	Dieldrin	PCB	Total Hg	Methyl Hg
1	June 17	♂	85.5	26.3	Blubber	96.80	20.66	4.03	0.31	11.98	36.98	0.15	35.12	0.027	–
					Muscle	0.85	0.14	0.04	trace	0.04	0.22	trace	0.50	0.360	–
					Liver	2.80	0.45	0.81	N.D.	0.11	0.87	trace	1.00	1.500	0.300 (20.0%)
					Cerebrum	8.90	0.23	0.07	N.D.	0.04	0.34	trace	0.62	0.110	–
2	July 13	♂	95.0	29.5	Blubber	85.70	52.51	6.00	trace	19.95	78.46	0.20	120.19	0.037	–
					Muscle	0.55	0.09	0.01	N.D.	0.02	0.12	trace	0.28	1.040	–
					Liver	3.60	1.15	0.19	N.D.	0.14	1.48	N.D.	2.50	7.900	1.010 (12.7%)
					Cerebrum	10.00	0.70	trace	N.D.	trace	0.70	trace	1.20	0.280	–
3	June 16	♂	130.0	72.6	Blubber	94.50	53.86	21.29	N.D.	64.02	139.15	0.38	240.21	0.064	–
					Muscle	0.66	–	–	–	–	–	–	–	0.550	–
					Liver	3.50	1.23	0.44	N.D.	0.26	1.93	0.04	6.00	2.980	–
					Cerebrum	9.50	0.51	0.19	N.D.	0.19	0.89	0.01	2.80	0.230	–
4	July 13	♀	88.0	27.2	Blubber	93.10	27.71	3.20	N.D.	16.00	46.92	trace	27.93	0.038	–
					Muscle	0.63	0.09	0.01	N.D.	0.01	0.11	trace	0.25	0.210	–
					Liver	2.60	0.40	0.25	N.D.	trace	0.65	trace	0.68	0.520	–
					Cerebrum	8.20	0.21	N.D.	N.D.	N.D.	0.21	N.D.	0.33	0.050	–
5	Aug. 27	♀	88.5	–	Blubber	89.80	14.86	1.14	trace	9.23	25.26	0.35	32.00	0.028	–
					Muscle	0.45	0.09	0.01	N.D.	N.D.	0.10	trace	0.10	0.760	–
					Liver	2.30	0.20	0.18	N.D.	0.11	0.38	trace	0.10	1.910	0.700 (36.6%)
					Cerebrum	8.10	0.38	0.07	N.D.	N.D.	0.45	N.D.	N.D.	0.230	–
6	June 15	♀	118.0	55.8	Blubber	92.20	32.10	11.20	0.09	25.05	65.54	0.06	99.78	0.087	–
					Muscle	2.20	0.04	trace	N.D.	trace	0.04	trace	0.20	1.540	–
					Liver	0.95	0.05	0.02	N.D.	0.02	0.09	trace	0.40	3.820	0.770 (20.1%)

TABLE 2. DDT, PCB, and total Hg in harbour seals (*Phoca vitulina*) from Grand Manan Island, and Deer Island (#9 only), New Brunswick, in 1971. All results expressed as parts per million of thawed tissue. N.D. = "not detected."

Specimen no.	Date taken	Sex	Length (*cm*)	Weight (*kg*)	Tissue	% fat in tissue	*p,p'*-DDE	*p,p'*-DDD	*o,p'*-DDT	*p,p'*-DDT	ΣDDT	Dieldrin	PCB	Total Hg	Methyl Hg
7	Aug. 11	♂	30[a]	20[a]	Blubber	88.50	33.00	1.41	N.D.	17.70	52.10	0.27	43.00	0.061	–
					Muscle	1.00	0.27	0.04	N.D.	0.01	0.32	N.D.	0.30	0.350	–
					Liver	5.20	1.04	0.73	N.D.	0.21	1.98	0.03	2.30	1.720	0.180 (10.5%)
					Cerebrum	7.40	0.44	0.03	N.D.	N.D.	0.47	N.D.	0.80	0.146	–
8	Aug. 11	♂	80[a]	20[a]	Blubber	90.10	21.62	0.36	N.D.	12.01	33.99	0.31	51.00	0.106	–
					Muscle	5.80	2.03	0.12	N.D.	0.62	2.77	0.03	5.10	0.550	–
					Liver	1.90	0.34	0.01	N.D.	N.D.	0.35	trace	0.80	3.170	0.340 (10.7%)
					Cerebrum	10.10	0.23	N.D.	N.D.	N.D.	0.23	N.D.	0.20	0.240	–
9	Aug. 7	♂	95.0	30[a]	Blubber	87.60	19.27	1.86	trace	8.00	29.13	1.16	63.00	0.060	–
					Muscle	1.40	0.32	0.01	N.D.	N.D.	0.33	0.01	0.50	0.380	–
10	Sept. 13	♂	127.5	60[a]	Cerebrum	10.3	0.31	0.03	N.D.	N.D.	0.34	0.02	0.50	0.760	–
11	Sept. 13	♂	144.8	90[a]	Muscle	1.7	0.19	0.02	N.D.	0.01	0.22	trace	0.40	0.590	–
					Liver	6.1	1.79	0.47	N.D.	N.D.	2.26	0.02	4.50	13.100	0.300 (2.3%)
					Cerebrum	9.4	0.29	0.01	N.D.	N.D.	0.30	trace	0.70	0.290	–
12	Aug. 11	♀	125[a]	55[a]	Blubber	89.0	4.90	0.18	N.D.	3.56	8.64	0.04	7,10	0.036	–
					Muscle	2.5	0.17	0.01	N.D.	0.05	0.23	trace	0.02	0.160	–
					Liver	4.1	0.22	0.13	N.D.	N.D.	0.35	trace	0.13	50.900	0.930 (1.8%)
					Cerebrum	5.4	0.01	N.D.	N.D.	N.D.	0.01	N.D.	0.01	0.380	–

[a]Approximately.

methylmercury increased, it appeared to do so at a much slower rate than the total mercury. Such an increase in methyl with total mercury is not clearly apparent in the present limited data. Since the migratory or local movements of the harbour seal in this region are not known, variation in individual exposure cannot be estimated and more detailed discussion is precluded.

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